Voluntary Running and Estrous Cycle Modulate ΔFOSB in the Suprachiasmatic Nucleus of the Wistar Rat

AYANO SHIBA

MARENE H. HARDONK

EWOUT FOPPEN

TESS KOOL

SUSANNE E. LA FLEUR

PAUL J. LUCASSEN

CHUN-XIA YI

DIRK JAN STENVERS

JORAM D. MUL**

ANDRIES KALSBEEK**

*Author affiliations can be found in the back matter of this article

**Shared senior authors

ABSTRACT

The hypothalamic suprachiasmatic nucleus (SCN), the circadian pacemaker of the mammalian brain, integrates both environmental and endogenous information to modulate various physiological and behavioral processes. Both light and physical activity entrain SCN circadian rhythmicity, but the underlying molecular mechanisms for physical activity remain elusive.

Repetitive neuronal stimulation results in accumulation of the stable transcription factor ΔFOSB, that has been implicated in long-term brain plasticity, altered neuronal excitability, and changes in behavior. In rodents, voluntary wheel running (VWR) mimics aspects of exercise training and increases ΔFOSB in several brain regions. Whether VWR also alters ΔFOSB in the SCN is unexplored.

Here, young-adult male and female Wistar rats were housed sedentary or allowed to run for four weeks followed by quantification of ΔFOSB in the SCN. VWR lowered SCN ΔFOSB-positive cell numbers in males and females compared to sedentary housing. Total running distance did not correlate with ΔFOSB suppression. Analysis taking estrous cycle into account revealed that ΔFOSB-positive cell numbers were cyclic in sedentary females, being lowest during proestrus and highest during diestrus. Remarkably, this cyclicity was absent in runners, where ΔFOSB-positive cell numbers remained comparable to those observed during proestrus in sedentary controls. Finally, estradiol replacement following ovariectomy in sedentary females lowered SCN ΔFOSB-positive cell numbers.

Thus, VWR and estrous cycle, via, at least in part, estradiol, modulate SCN ΔFOSB. Given its role in long-term plasticity and behavioral adaptations, ΔFOSB may provide a molecular link between VWR and/or estrous cycle and the output of the SCN and its related behavioral adaptations.

INTRODUCTION

The suprachiasmatic nucleus of the hypothalamus (SCN) is the circadian pacemaker of the mammalian brain. It enforces daily rhythms in several physiological and behavioral processes, including those in energy homeostasis, various hormones, sleep/wake cycles and physical activity. The SCN integrates external and internal information to entrain its own circadian rhythmicity to the 24h environmental light/dark cycle. Important entrainers, or zeitgebers, of the SCN are exposure to light, consumption of calories, and physical activity [1, 2].

A commonly used behavioral paradigm to monitor daily SCN output in rhythmicity is voluntary wheel running (VWR). In rodents, VWR is a self-reinforcing behavior, that can profoundly impact plasticity of the brain [3–8]. Access to a running wheel can *e.g.* affect circadian rhythmicity and output of the SCN, depending on the amount, intensity and timing of VWR [9–11]. Moreover, VWR, when performed during the active (*i.e.* dark) phase of rats, enhances the amplitude of SCN oscillations [12]. Next to VWR, another cyclic input to the SCN is the female hormonal cycle and

locomotor activity patterns are e.g. influenced also by the estrous cycle. Although several studies have investigated the impact of VWR on the central (SCN) and peripheral clocks [12–14], the underlying molecular mechanisms as to how VWR can impact the SCN, and whether this occurs in a sex-dependent manner, remain poorly understood.

ΔFOSB is a highly stable transcription factor that mediates activity-dependent gene expression and is involved in long-lasting molecular and structural adaptations in neuronal circuits involved in reward processing and addiction, memory, and stress resilience [15–20]. ΔFOSB is a family member of the FOS family of immediate early gene activator protein-1 (AP-1) transcription factors and is generated via alternative splicing of the parent *Fosb* mRNA. ΔFOSB regulates gene expression via self-assemblies and via the formation of heterodimers with JUN proteins, such as JUND, to form AP-1 transcription complexes [16, 21–23]. Due to several structural modifications, ΔFOSB has a remarkable long half-life compared to other FOS-related transcription factors and can persist for days or weeks in neurons where it accumulates when they are stimulated repetitively [24–29].

Given its role in reward, memory, and stress, studies that investigated ΔFOSB have so far focused on striatal, cortical or hippocampal brain regions [15, 30–32]. However, FOSB and ΔFOSB are also expressed in the hypothalamus, where high levels are observed in the SCN [33–37]. Although these studies have revealed differential regulation of FOS family proteins in rodent SCN in response to light, it is currently unclear if ΔFOSB is involved in the effects of VWR on the circadian timing system. Due to its high stability, ΔFOSB can regulate gene expression and may thus modulate physiological processes and neuronal function for an extended timeframe. Whether sex differences exist was unclear and we therefore here investigated if four weeks of voluntary running would modulate SCN ΔFOSB levels, both in male and female Wistar rats.

MATERIALS AND METHODS

ETHICS APPROVAL STATEMENT
All experimental procedures were performed in accordance with the European guidelines for laboratory animals (EU directive 2010\63\EU) and approved by the Dutch Central Committee for Animal Experiments (CCD; AVD11800202316744, AVD80100202216157 and AVD8010020172424) and the Agency for Animal Welfare (IvD) of the Netherlands Institute for Neuroscience (NIN; Royal Dutch Academy of Sciences). To minimize general distress, rats were housed in cages with corncob bedding and cage enrichment [an aspen wood gnawing stick (Technilab-BMI) and a PVC shelter (Bio Services)]. Furthermore, rats were housed in open cages and in the same experimental room, which enables them to smell and hear each other. This was done in an attempt to counterbalance the solitary housing during the experiment, which was necessary to adequately measure individual running distance and caloric intake.

ANIMAL COHORTS
Housing conditions
A total of 28 male and 76 female Wistar WU rats [Crl:WI(WU), Charles River, Germany] were used for these experiments. All rats were housed in a temperature- (21–23°C), humidity- (40–60%) and light-controlled experimental room [12:12h light/dark cycle; 280 (±80) Lux (lights on), <5 Lux (lights off); with lights on at Zeitgeber time 0 (ZT0) and lights off at ZT12] in the NIN animal facility. A sound system continuously played soft radio music during the experiments to provide background noise. To recover from transport stress and habituate to the animal facility, rats were group-housed (4/cage) in a polycarbonate type 4 cage [530(l) × 330(w) × 200(h) mm; 1815 cm2; Plexx] for

one week upon arrival at the NIN. During acclimatization and experiments, rats had *ad libitum* access to a bottle of tap water and an irradiated nutritionally complete high-carbohydrate diet (Teklad global diet 2918, 24% kcal from protein, 58% kcal from carbohydrate, and 18% kcal from fat, 3.1kcal/g, Envigo).

Primary male and female running cohorts for SCN ΔFOSB analysis
After 6–8 days of acclimatization to the NIN animal facility, male and female rats (weighing 255–270 grams and 170–200 grams, respectively, upon arrival; approximately 8–9 weeks old) were individually housed and randomly assigned to one of two experimental groups: sedentary controls (male, n = 14; female, n = 12) were housed without access to a running wheel in a polycarbonate type 3H cage [375 (l) × 215 (w) × 180 (h) mm)], whereas runners (male, n = 14; female, n = 12) were housed in custom-made cages [522 (w) × 582 (l) × 412 (h) mm] with free access to a stainless-steel vertical wheel (width: 11 cm, diameter: 35.6 cm, 1.1m/revolution; model 80850MS, Campden Instruments) for 29 days. Wheel revolutions were continuously registered every second using in-house developed software. The experimental groups were body weight-matched at the start of the experiment and body weight was measured every seven days. Caloric and water intake was measured every 2–3 days. Twenty-four hours prior to the end of the experiment, running wheels were blocked for runners to make sure any residual wheel running-induced full-length FOSB protein had been degraded at the time of sacrifice, and all remaining immunoreactivity primarily reflects ΔFOSB [38]. This procedure was necessary as the rabbit monoclonal antiserum that was used for the detection of the ΔFOSB signal was raised against the N-terminal region of FOSB, thereby recognizing both FOSB and ΔFOSB (5G4; #2251, Cell Signaling Technology). For female rats, a vaginal smear was taken before perfusion to determine estrous stage (*i.e.* proestrus, estrus, metestrus, and diestrus) based on the ratio of nucleated epithelial cells, cornified cells, and leukocytes present in vaginal smears. After 29 days of running, rats were deeply anesthetized between ZT5 and ZT7, *i.e.* in the middle of their inactive phase, with an overdose of sodium-pentobarbital and transcardially perfused with ice-cold 0.9% saline followed by ice-cold 4% paraformaldehyde in 0.1M phosphate-buffered saline (PBS; pH 7.4). Brains were removed and stored in 4% paraformaldehyde in PBS at 4°C for at least 24 hrs. Thereafter, for cryoprotection, brains were transferred to 30% sucrose in 1X tris-buffered saline (TBS; 50 mM Tris–Cl, 150 mM NaCl; pH 7.6) with 0.05% sodium azide and stored at 4°C until further processing. Dehydration was considered complete when the brains had fully sunk to the

bottom of the container, which required at least 24 hours. Coronal brain sections (35 µm) were cut on a cryostat and stored in cryoprotectant medium (30%v/v glycerol, 30%v/v glyceraldehyde and 40%v/v 1X TBS) at −20°C. Coronal brain slices from Bregma −0.48 mm to −0.96 mm were then used for quantification of ΔFOSB levels in the (rostral to caudal) SCN (Supplemental Fig. 1).

Female running cohort for verification of SCN ΔFOSB analysis

Data from a second and independent female running cohort (experiment performed in a different room at the NIN with slightly smaller wheels) was used to confirm quantification of SCN ΔFOSB levels after voluntary running. After seven days of acclimatization to the NIN animal facility, female rats (weighing 170–200 grams upon arrival; approximately 8–9 weeks old) were individually housed without access to a running wheel (*i.e.* sedentary; n = 11) in a polycarbonate type 3H cage [375 (l) × 215 (w) × 180 (h) mm)] or housed with free access to a stainless steel vertical running wheel (*i.e.* runners: female: n = 11; width: 10 cm, diameter: 34 cm, 1.068m/revolution) in a custom-made cage (422 (l) × 422 (w) × 475 (h) mm) for 28 days. Wheel revolutions were continuously registered using an in-house developed Cage Registration Program (Dep. Biomedical Engineering, UMC Utrecht, The Netherlands). Two male transgenic ChAT::*Cre* Long-Evans rats were housed in the same room from the start of the experiment in an attempt to synchronize female estrous cycles. Twenty-four hours prior to the end of the experiment, running wheels were blocked for runners to make sure any residual wheel running-induced full-length FOSB protein had been degraded at the time of sacrifice, and all remaining immunoreactivity primarily reflects ΔFOSB [38]. Rats were killed by perfusion, brains were removed and processed in the same manner as for the primary running cohorts, and a vaginal smear was taken to determine estrous stage.

Female verification cohort for ΔFOSB analysis during estrous cycle

A third and independent female sedentary cohort was used to further confirm quantification of SCN ΔFOSB levels during the estrous cycle. After five days of acclimatization to the NIN animal facility, female rats (n = 12; weighing 195–225 grams upon arrival; approximately 10–11 weeks old) were pair-housed and a vaginal smear was taken every day at ZT2 to determine estrous stage. After completing 2–3 estrous cycles, rats were killed in the proestrus or diestrus stage by perfusion that same day between ZT5 and ZT7, and brains were removed and processed similar to the primary running cohorts. Data from two rats (one

in proestrus stage and one in diestrus stage) had to be excluded from analysis due to missing brain sections.

Female cohort for SCN ΔFOSB analysis following ovariectomy and estradiol replacement

A fourth female cohort was used to determine if estradiol replacement in ovariectomized female rats modulates SCN ΔFOSB. After 5–7 days of acclimatization to the NIN animal facility, 16 pair-housed female rats (weighing 170–200 grams upon arrival; 8–9 weeks old) were anesthetized with inhalation isoflurane (3–5% induction, followed by 1.5–2,5% maintenance) and ovaries were removed through two 1.5-cm lateral dorsal incisions in the retroperitoneal region. For pain relief, rats were injected with Meloxicam (1 mg/kg; Metacam, Hyperdrug C.O., United Kingdom) once before and once immediately after the ovariectomy surgery. In addition, Rimadyl (Carprofen, 0.6–1.5 mg/rat/day; Zoetis, the Netherlands) was added to the drinking water starting during the lights-on phase of the surgery day and provided for 72h. Subsequently, the drinking water was replaced with new drinking water containing either vehicle (final concentration: 0.031% ethanol; n = 8) or estradiol (875µg/L 17β-estradiol, #E8875, Sigma Aldrich; dissolved in 0.031% ethanol; n = 8) [39, 40]. Rats were pair-housed within treatment condition (vehicle or estradiol). After 23 days of estradiol replacement via the drinking water, rats were killed by perfusion between ZT5 and ZT7, brains were removed and processed in the same manner as for the primary running cohorts, and a vaginal smear was taken to determine estrous stage. In addition, uteri were isolated and weighed to determine the effects of estradiol replacement on uterus size. Data from one vehicle-treated animal had to be excluded from analysis due to missing brain sections.

IMMUNOCYTOCHEMISTRY TO DETERMINE THE ESTROUS STAGE

Vaginal epithelial cells were collected with sterile plastic swabs (#861.562.010, Sarstedt BV), placed on glass slides (#631-0457, VWR), and dried overnight at room temperature, or at 55°C for 30 min when determination of estrous stage was done right before killing of the rats. After drying, smears were stained with Giemsa solution (#48900-100ML-F, Merck) for 10 min, washed 3x with 1X TBS, dried overnight at room temperature, or at 55°C for 30 min when determination of estrous stage was done right before killing of the rats, and estrous stage was determined using a microscope (DM2000, Leica).

SINGLE-LABELING IMMUNOHISTOCHEMISTRY

ΔFOSB immunoreactivity was detected using a rabbit monoclonal antiserum raised against the N-terminal

region of FOSB that recognizes both FOSB and ΔFOSB, as mentioned above (5G4; #2251, Cell Signaling Technology). ΔFOSB-like staining was revealed by use of the avidin-biotin peroxidase method. Coronal brain slice sections were pre-mounted on the Superfrost ++ glass slides (Menzel), washed 3x in 1X TBS before antigen retrieval by incubation for 1.5h with Tris-citrate buffer (pH 8.0) at 95℃. Sections were then washed 3x in 1X TBS, and blocked for 45 min in 3% skim milk (Campina) in 1X TBS. Thereafter, sections were incubated for 1 hr in a humidified chamber at room temperature and then overnight at 4℃ with Supermix (0.25% w/v gelatin, 0.5% v/v Triton X-100, in 1x TBS, pH 7.6) containing anti-FosB(N-terminus) antibody (1:1000; 5G4; #2251, Cell Signaling Technology). Sections were washed with TBS and incubated for 1 hr at room temperature with 10 mM sodium phosphate, pH 7.8, 0.15 M NaCl, 0.08% sodium azide, 3 mg/ml bovine serum albumin containing biotinylated goat anti-rabbit IgG (1:400; H+L; BA9200; Vector Laboratories, Burlingham) diluted in Supermix. Sections were washed with TBS and incubated for 1 hr at room temperature with avidin-biotin complex (1:800; Vectastain Elite ABC HRP Kit, Vector Laboratories) diluted in Supermix. Peroxidase activity was visualized by reaction with 0.05M TB (pH 7.6) containing 0.05% w/v diaminobenzidine (Vector Laboratories), 0.23% w/v nickelammoniumsulphate (Merck) and 0.01% w/v H_2O_2 (Merck). The reaction was stopped by dilution in tap water. Sections underwent dehydration with increasing concentrations of ethanol and 100% Xylene, and coverslipped with Entellan (Merck).

Sections were scanned using a Zeiss Axio Scan.Z1 slide scanner (Carl Zeiss AG, Oberkochen, Germany). Bregma of brain slices was determined based on the optic nerve lengths and the morphological features of landmarks and other brain regions in each section. Per rat, five equally spaced SCN sections (Bregma –0.48, –0.60, –0.72, –0.84, and –0.96) were used to quantify the numbers of ΔFOSB-immunoreactive nuclei within the SCN using QuPath Cell Detection [41]. The experimenter (AS) was unaware of the treatment information to avoid bias during analysis.

DOUBLE-LABELING IMMUNOHISTOCHEMISTRY

To link ΔFOSB expression to specific SCN cell types, immunofluorescence was used to double-label for ΔFOSB [1:100; a custom-made Alexa Fluor 488 pre-conjugated FOSB (5G4) rabbit monoclonal antibody, Cell Signaling Technology] and for vasoactive intestinal peptide (VIP; 1:500; Viper, #RRID: AB_2513212 rabbit polyclonal antibody developed in-house at the NIN) or arginine vasopressin (AVP; 1:500; Truus 86, #RRID: AB_2313977, rabbit polyclonal antibody developed in-house at the NIN). Sections were pre-mounted on the Superfrost ++ glass slides (Menzel), washed 3x in 1X TBS before antigen

retrieval by incubation for 1.5 h with Tris-citrate buffer (pH 8.0) at 95℃. Sections were then washed 3x in 1X TBS, and blocked for 1hr in 10% normal donkey serum in PBS. Sections were incubated for 1 hr in a humidified chamber at room temperature and then overnight at 4℃ with supermix (0.25% w/v gelatin, 0.5% v/v Triton X-100, in 1X TBS, pH 7.6) containing the primary antibodies. Sections were rinsed with 1X TBS, and incubated for 1hr at room temperature with 5 mM sodium azide and 1X PBS (pH 7.5) containing Alexa Fluor 594 goat anti-rabbit IgG (1:400, Invitrogen; for VIP and AVP) diluted in supermix. After counterstaining with 4,6-diamidino-2-phenylindole (DAPI, 1:2000, Thermo Fisher), slides were coverslipped with mounting medium (Vectashield Vibrance, Vector Laboratories). Photomicrographs for localization of protein expression were obtained using a confocal microscope (Leica TCS SP5 ll) equipped with an inverted DMi6000 with a 40x objective (HC PL APO CS2 40x1.4 oil).

STATISTICS

Data are presented as mean ± SEM. Two-group comparisons were performed by two-tailed Student's t-test. Assessment of effects in experiments involving several conditions was performed using two-way analysis of variance (ANOVA), with repeated measures where applicable, followed, when appropriate, by Tukey HSD or Sidak's post hoc tests to adjust for multiple comparisons. A *P* value <0.05 was considered significant. See figure legends for statistical details of individual experiments, including statistical tests used, *t*, *P*, F-values, and number of subjects or samples tested. GraphPad Prism v10 was used to generate graphs and perform statistical analysis.

RESULTS

VOLUNTARY RUNNING BEHAVIOR AND PHYSIOLOGICAL EFFECTS IN MALE AND FEMALE WISTAR RATS

During the first two weeks of running, both male and female runners showed a steady increase (*i.e.* the acquisition phase) followed by a stabilization of daily running distances around 5 km/day for the male and 11 km/day for the female runners (*i.e.* the maintenance phase; Figure 1a,d). In line with the nocturnal nature of rats, most of the running was performed during the dark phase (Figure 1b,e). After 29 days of running, total running distances were around 139 ± 67 km for the male runners and around 242 ± 72 km for the female runners (Figure 1c,f). Both sedentary and running male and female rats gained body weight (Figure 1g–j), but both running groups less so compared to their respective sedentary controls (Figure 1g–j).
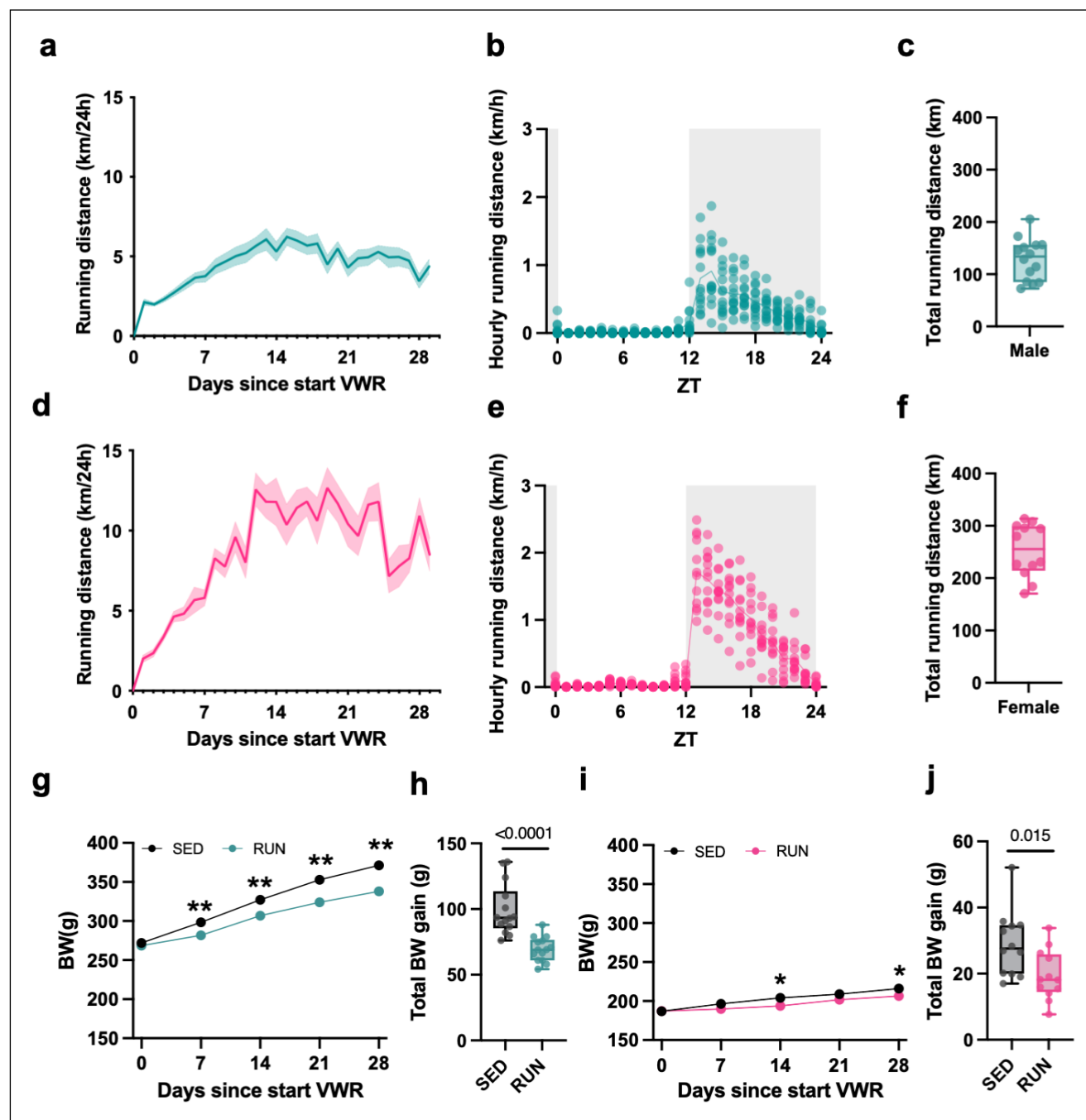
Figure 1 Voluntary running behavior and impact on body weight. (a) Daily running distance, **(b)** daily running patterns and **(c)** total running distance of male runners during 29 days of running. **(d)** Daily running distance, **(e)** daily running patterns and **(f)** total running distance of female runners during 29 days of running. **(g)** Body weight (BW) evolution (*time × housing* interaction, $F_{(4, 104)} = 20.89$, $P < 0.0001$; Tukey post hoc: *$P < 0.05$, **$P < 0.01$, SED *versus* RUN) and **(h)** total BW gain ($t = 5.158$, $df = 26$, $P < 0.0001$ versus SED) of male runners (RUN) and sedentary (SED) controls. **(i)** BW evolution (*time × housing* interaction, $F_{(4, 88)} = 5.14$, $P = 0.0009$; Sidak post hoc: *$P < 0.05$, SED *versus* RUN) and **(j)** total body weight gain ($t = 2.646$, $df = 22$, $P = 0.0147$ versus SED) of female runners (RUN) and sedentary (SED) controls. Data are presented as the mean ± S.E.M or as box plots indicating the median (line), the interquartile range, and the minimum to maximum values of the data distribution, with dots representing individual rats. a,b,c,g,h: $n = 14$/group; d,e,f,i,j: $n = 12$/group.

VOLUNTARY RUNNING MODULATES SCN ΔFOSB IN MALE AND FEMALE WISTAR RATS

We next quantified ΔFOSB expression in the SCN of male and female runners and sedentary controls after 29 days of voluntary running. Brain slices between bregma –0.96 mm and –0.48 mm were used to quantify the numbers of cells expressing ΔFOSB protein in the (rostral to caudal) SCN (Supplemental Figs. 1a,b). In both male and female rats, the number of SCN ΔFOSB-positive cells was lowest at the rostral and caudal SCN tails and was highest around bregma –0.72 mm (Figure 2a–c; Supplemental Figs. 1a,b).
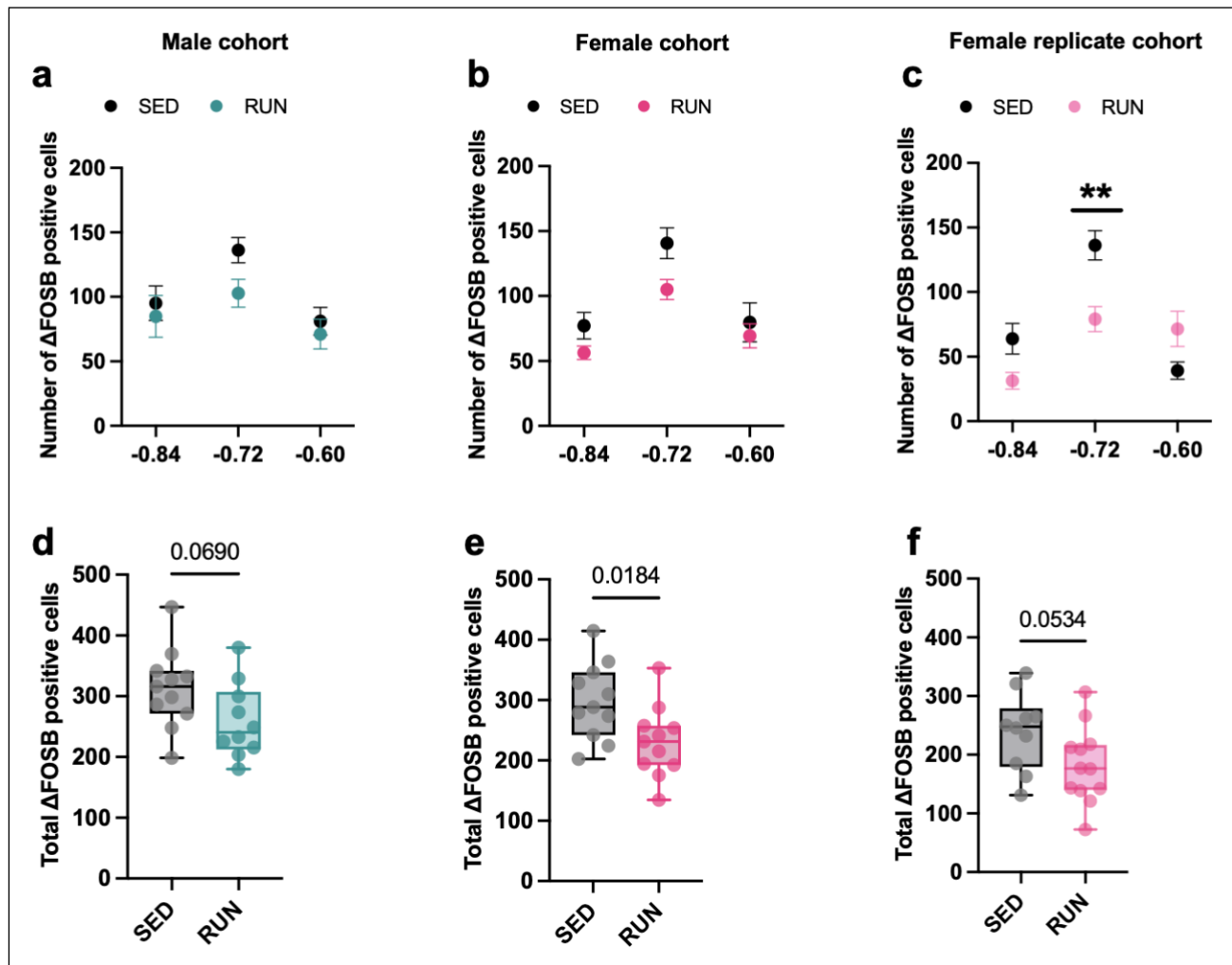
Figure 2 Long-term voluntary running lowers SCN ΔFOSB. (a–c) Quantification of ΔFOSB-positive cell numbers in the SCN (between bregma –0.6 mm and –0.84 mm) of **(a)** male runners (RUN) and sedentary (SED) controls (running for 29 days; *bregma × housing* interaction, $F_{(2, 38)}$ = 0.5658, P = 0.5726; main effect of *bregma*, $F_{(1.65, 31.34)}$ = 6.093, P = 0.0086; main effect of *housing*, $F_{(1, 19)}$ = 3.715, P = 0.069), **(b)** female runners (RUN) and sedentary (SED) controls (*bregma × housing* interaction, $F_{(2, 40)}$ = 0.7783, P = 0.4660; main effect of *bregma*, $F_{(1.256, 25.11)}$ = 17.55, P < 0.0001; main effect of *housing*, $F_{(1, 20)}$ = 6.586, P = 0.0184), and in the SCN of **(c)** an independent replication cohort of female runners (RUN) and sedentary (SED) controls (running for 28 days; *bregma × housing* interaction, $F_{(2, 40)}$ = 10.83, P = 0.0002, Sidak's post hoc: **P = 0.0035, SED$^{-0.72}$ *versus* RUN$^{-0.72}$; main effect of *bregma*, $F_{(1.912, 38.24)}$ = 21.57, P < 0.0001; main effect of *housing*, $F_{(1, 20)}$ = 4.214, P = 0.0534). **(d–e)** Total average number of ΔFOSB-positive cells in the SCN (between bregma –0.6 mm and –0.84 mm) of **(d)** male runners (RUN) and sedentary (SED) controls (t = 1.927, df = 19, P = 0.069 versus SED), **(e)** female runners (RUN) and sedentary (SED) controls (t = 2.566, df = 20, P = 0.0184 versus SED), and in the SCN of **(f)** an independent replication cohort of female runners (RUN) and sedentary (SED) controls (t = 2.053, df = 20, P = 0.0534 versus SED). Data are presented as the mean ± S.E.M **(a–c)** or as box plots indicating the median (line), the interquartile range, and the minimum to maximum values of the data, with dots representing individual rats **(d–f)**. a,d: *n* = 10–11/group; b,e: *n* = 10–12/group, c,f: *n* = 11/group.

Voluntary running for 29 days resulted in a trend for lower ΔFOSB-positive cell numbers in the SCN of male runners compared to male sedentary controls (P = 0.069; Figure 2a,d). In females, voluntary running for 29 days did significantly lower SCN ΔFOSB-positive cell numbers in runners compared to sedentary controls (P = 0.0184; Figure 2b,e).

We then used data from an independent, yet comparable, cohort of female runners and sedentary controls to replicate and confirm these observations. In this replication cohort, voluntary running for 28 days resulted in a trend for lower ΔFOSB-positive cell numbers in the SCN of runners compared to sedentary controls (P = 0.053; Figure 2c,f). Finally, we assessed if ΔFOSB-positive cell numbers in the SCN were related to total running distances, but we observed no significant correlations for the primary male nor the female cohort (Supplemental Figs. 2a,b). Thus, long-term voluntary running suppresses the number of ΔFOSB-positive cells in the SCN of both male and female rats, an effect that was more prominent in females than in males and was independent of total distance ran.

SCN ΔFOSB CORRELATES WITH ESTROUS CYCLE IN SEDENTARY FEMALE WISTAR RATS

Female locomotor activity patterns are not only strongly influenced by the circadian clock, but also by the estrous cycle. To explore whether SCN ΔFOSB levels fluctuate with the estrous cycle, data from female sedentary controls of the primary running cohort and the female running replication cohort were stratified with respect to estrous stage (*i.e.* proestrus, estrus, metestrus, and diestrus) at time of death (Supplemental Fig. 3a). Analysis of ΔFOSB-positive cells in the SCN of sedentary female controls revealed a cyclic pattern, being lowest in the proestrus phase and highest in the diestrus phase, whereas this cyclicity was absent in female runners (Figure 3a). Thus the general decrease in SCN ΔFOSB in female runners compared to sedentary controls (Figure 2b,c,e,f) appears to be primarily driven by a difference during the diestrus phase (Figure 3a). We then used data from a third cohort of only female sedentary controls to replicate and confirm these observations. The estrous cycle of these female sedentary rats was monitored daily (Supplemental Fig. 3b), and rats were killed after 2–3 complete cycles when being either in their proestrus or diestrus phase. Analysis of ΔFOSB- positive cells in the SCN in this third cohort of only female sedentary controls again revealed a cyclic pattern, being lowest in the proestrus phase and highest in the diestrus phase (Figure 3b). Thus, SCN ΔFOSB levels correlate with the estrous cycle in sedentary female Wistar rats, and this cyclicity was severely blunted in female runners.

ESTRADIOL REPLACEMENT FOLLOWING OVARIECTOMY MODULATES SCN ΔFOSB IN SEDENTARY FEMALE WISTAR RATS

Circulating estrogenic hormones, including estradiol, rhythmically fluctuate over the reproductive cycle in mammalian females. Having established a correlation between SCN ΔFOSB and estrous cycle in the sedentary condition, we next tested if replacing estradiol in an ovariectomy background directly modulates SCN ΔFOSB. To do this, sedentary pair-housed female rats were ovariectomized and treated with vehicle or estradiol for 23 days via the drinking water. Daily water intake (measured per cage) indicated that estradiol consumption fluctuated within the range of 60–90 μg/kg body weight/day/ animal throughout the experiment (Figure 4a,b). Estradiol replacement via the drinking water following ovariectomy was successful, as body weight gain was significantly lower in estradiol-treated rats compared to vehicle-treated controls (Figure 4c). Secondly, estradiol replacement following ovariectomy increased uterus weight compared to vehicle treatment (Figure 4d,e). Thirdly, vaginal smears taken at the end of the experiment confirmed that vehicle-treated females displayed "diestrus-stage like" characteristics, whereas estradiol-treated females
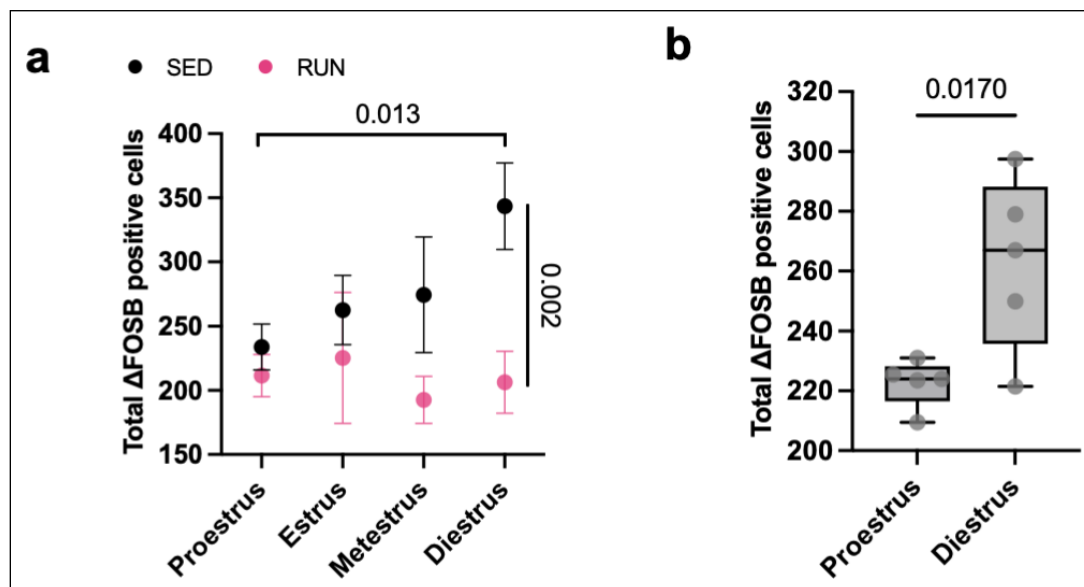

Figure 3 Estrous stages modulate SCN ΔFOSB. (a) Quantification of ΔFOSB-positive cell numbers in the SCN (between bregma –0.6 mm and –0.84 mm) of female runners (RUN) and sedentary (SED) controls divided by estrous stage during collection of brain tissue (main effect of *housing*, $F_{(1, 17)} = 9.213$, $P = 0.0075$; Tukey post hoc: $P = 0.002$, SED[Diestrus] *versus* RUN[Diestrus]; $P = 0.013$, SED[diestrus] *versus* SED[proestrus]). **(b)** Quantification of ΔFOSB-positive cell numbers in the SCN (between bregma –0.6 mm and –0.84 mm) of an independent replication cohort of female sedentary controls killed at proestrus or diestrus after completing at least two estrous cycles (t = 3.001, df = 8, $P = 0.0170$ *versus* diestrus). Data are presented as the mean ± S.E.M (a) or as box plots indicating the median (line), the interquartile range, and the minimum to maximum values of the data, with dots representing individual rats (b). (a) proestrus: $n = 9$–10/group; estrus: $n = 4$/ group; metestrus: $n = 3$–5/group; diestrus: $n = 4$–5/group; (b) proestrus: $n = 5$; diestrus: $n = 5$.
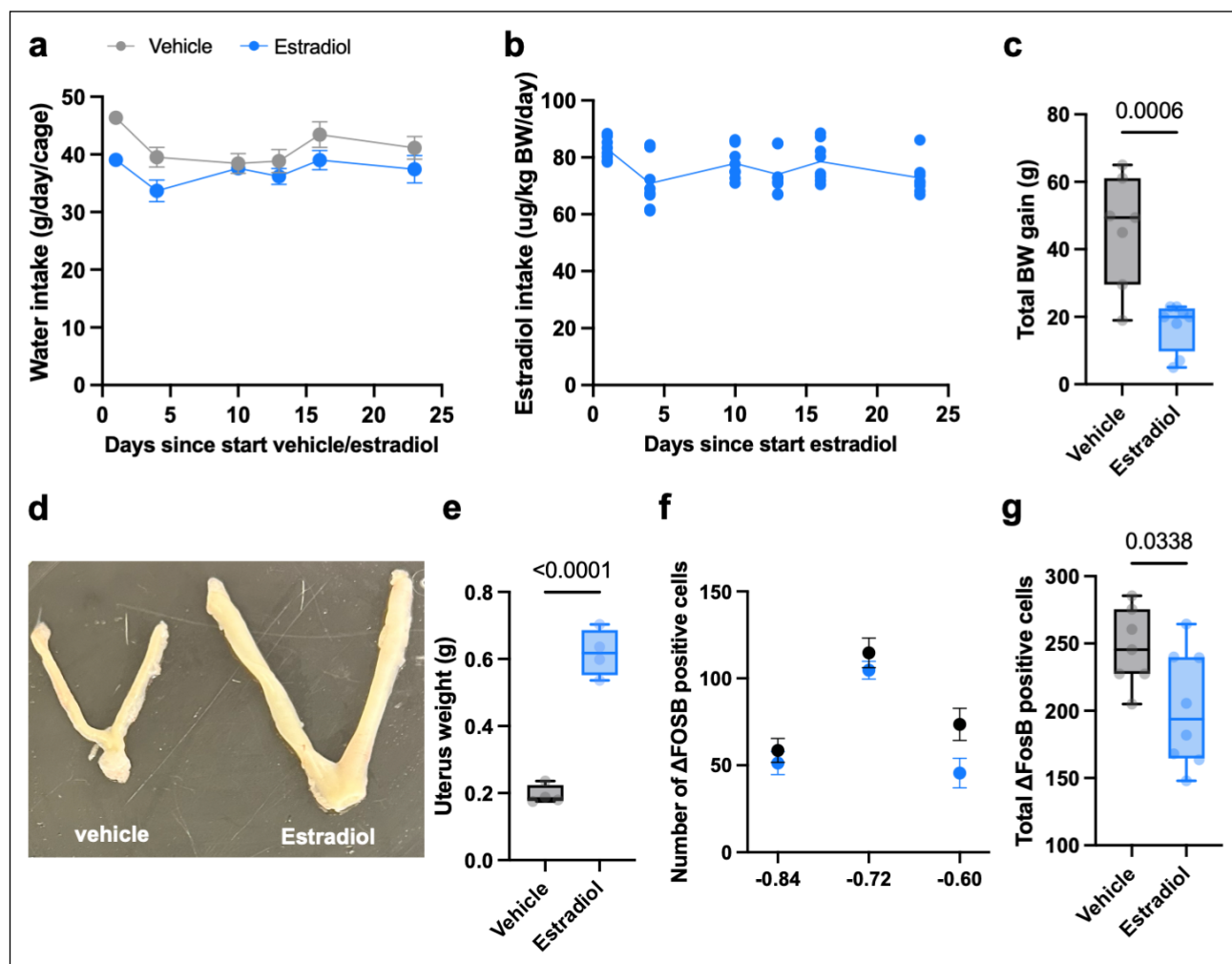
Figure 4 Estradiol replacement following ovariectomy lowers SCN ΔFOSB. (a) Average fluid intake (per cage) of water containing vehicle or estradiol during the 23 days of estradiol replacement. **(b)** Estradiol intake (per animal), normalized for body weight, calculated based on water intake. **(c)** Total body weight gain after 23 days of vehicle- or estradiol treatment. **(d,e)** Representative uteri (d; left, vehicle; right, estradiol) and uterus weight comparison **(e)** of vehicle- or estradiol-treated ovariectomized rats. **(f)** Quantification of ΔFOSB-positive cell numbers in the SCN (between bregma –0.60 mm and –0.84 mm) of ovariectomized rats with vehicle or estradiol (E2) replacement (*bregma × E2 treatment* interaction, $F_{(2, 26)} = 0.9877$, $P = 0.9877$; main effect of *bregma*, $F_{(1.533,19.93)} = 39.71$, $P < 0.0001$; main effect of *E2 treatment*, $F_{(1,13)} = 5.629$, $P = 0.0338$). **(g)** Total average number of ΔFOSB-positive cells in the SCN (between bregma –0.60 mm and –0.84 mm) following vehicle- or estradiol treatment ($t = 2.373$, df = 13, $P = 0.0338$). Data are presented as the mean ± S.E.M (d,f) or as box plots indicating the median (line), the interquartile range, and the minimum to maximum values of the data, with dots representing individual rats (b,e,g). (e) vehicle: n = 4; estradiol: n = 4, (a–c,f–g) vehicle: *n* = 7; estradiol: *n* = 8.

displayed "late-proestrus-stage like" characteristics (data not shown). Finally, estradiol replacement following ovariectomy lowered SCN ΔFOSB-positive cell numbers compared to vehicle treatment (Figure 4f,g). Thus, estradiol can directly modulate SCN ΔFOSB in ovariectomized sedentary female Wistar rats.

SCN ΔFOSB COLOCALIZES WITH VIP, BUT NOT WITH AVP

The SCN can be subdivided in two main subregions, a ventral core that contains the majority of VIP neurons, and a dorsal shell that contains the majority of AVP neurons

[42]. SCN ΔFOSB is predominantly expressed in neurons in the lateral part of the ventral core, but not in the dorsal shell (Supplemental Figs. 1a,b). We next used fluorescent double-labeling immunohistochemistry to determine if neuronal VIP and AVP populations in the SCN co-express ΔFOSB. In sedentary females, we observed several examples of SCN cells with co-expression of ΔFOSB and VIP, especially in the most lateral part of the SCN (Figure 5a$_i$–d$_i$). Such co-expression was not observed for ΔFOSB and AVP in *e.g.* the dorsal SCN (Figure 5a$_{ii}$–d$_{ii}$). Thus, ΔFOSB is predominantly expressed in the ventral core, but not dorsal shell, region and is co-expressed by VIP, but not AVP, cells.
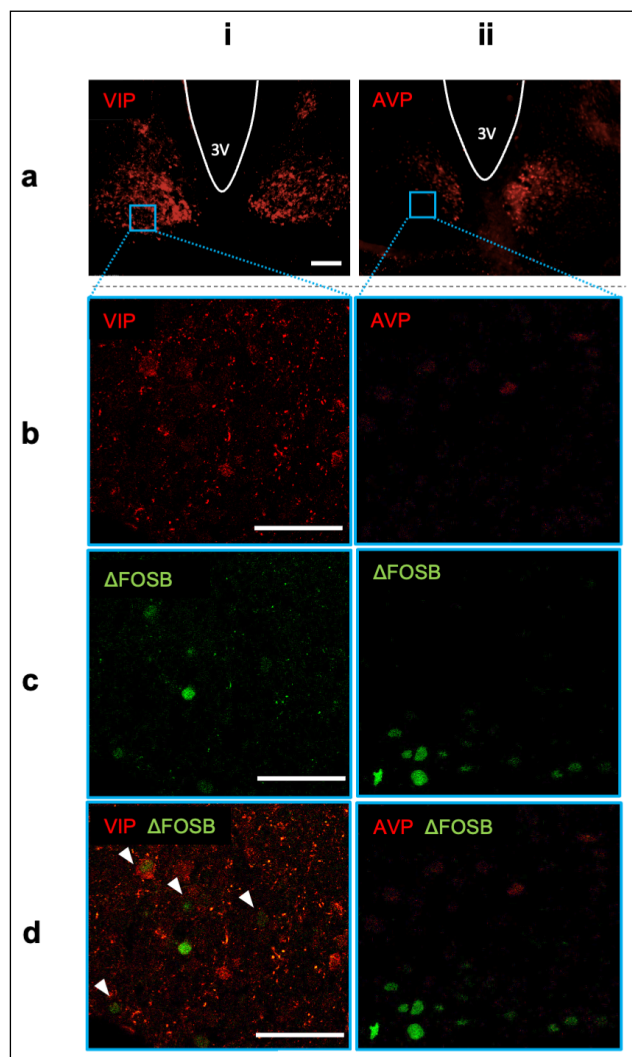
Figure 5 Co-expression of ΔFOSB with VIP, but not AVP, in the SCN. (a$_{i-ii}$) Examples of (a$_i$) VIP expression and (a$_{ii}$) AVP expression in the SCN (bregma –0.72 mm) of a female runner. **(b$_{i-ii}$)** Insets, shown as blue squares in a$_{i-ii}$, at 10x magnification for (b$_i$) VIP and (b$_{ii}$) AVP. **(c$_{i-ii}$)** ΔFOSB expression in insets, shown as blue squares in a$_{i-ii}$. **(d$_{i-ii}$)** Co-expression of (d$_i$) ΔFOSB and VIP and (d$_{ii}$) ΔFOSB and AVP in insets, shown as blue squares in a$_{i-ii}$. White arrowheads indicate co-expression of ΔFOSB and VIP. a$_{i-ii}$: scale bar = 100 μm; b$_{i-ii}$, c$_{i-ii}$, d$_{i-ii}$: scale bar = 50 μm.

DISCUSSION

Physical exercise has well-documented effects on circadian rhythmicity, but the underlying mechanisms in the brain remain to be fully elucidated. Here, we demonstrate that long-term voluntary running lowered ΔFOSB-positive cell numbers in the SCN in both male and female rats, an effect that was more prominent in females than in males and was independent of total running distance. Furthermore, the number of ΔFOSB-positive cells in the SCN fluctuated across the estrous cycle in female sedentary rats, with the highest levels being observed during diestrus

and the lowest levels during proestrus. This cyclicity was completely absent in female runners. Estradiol replacement following ovariectomy in sedentary females lowered SCN ΔFOSB-positive cell numbers. Finally, SCN ΔFOSB was predominantly expressed in neurons in the ventral core, but not dorsal shell, region of the SCN and was co-expressed to some degree with VIP, but not AVP.

A consistent finding in rodent studies is that long-term VWR increases ΔFOSB in several brain regions, including nucleus accumbens, dorsomedial striatum, dorsal raphe nucleus, hippocampal subregions, and medial prefrontal cortex [43–52]. Based on these observations, we hypothesized that VWR would also increase ΔFOSB in the SCN. Contrary to our hypothesis, we observed that voluntary running lowered ΔFOSB-positive cell numbers in the SCN of male and female rats compared to sedentary controls. To the best of our knowledge, one study has previously reported a decrease in FOSB/ΔFOSB following VWR, namely in the basolateral amygdala of individually-housed, running prairie voles compared to individually-housed sedentary prairie voles [51].

Using an inducible transgenic mouse model it was demonstrated that ΔFOSB activates or represses expression of many genes in a time-dependent manner [53]. This also occurs during physiological conditions, as accumulation of ΔFOSB, in striatal neurons following chronic amphetamine exposure or in dentate gyrus neurons following recurrent seizures, represses expression of *c-fos*, another Fos family gene and a classic marker for cellular activation, via epigenetic mechanisms [54, 55]. Furthermore, learning-mediated accumulation of hippocampal ΔFOSB inhibits production of cFOS in granule cells of the dentate gyrus, but not in neurons of the CA1, and is involved in memory processes [30]. Finally, viral-mediated overexpression of ΔFOSB decreases excitability of dorsal hippocampal CA1 neurons [56], consistent with the notion that target genes of ΔFOSB include critical regulators of glutamatergic synaptic strength, including certain AMPA-type glutamate receptor subunits (*e.g.* glutamate receptor 2), Ca2+/calmodulin-dependent protein kinase IIα, and cyclin-dependent kinase 5 [20, 57–61].

Taken together, these findings suggest that repression of SCN ΔFOSB by VWR might be mirrored by greater neuronal excitability, compared to sedentary controls, and a higher amplitude of neuronal activity rhythms. Potentially, this may sensitize the SCN, and especially the ventral core, to environmental changes in entrainers, like light. In rats living in the wild, an increased excitability of the SCN may facilitate responsiveness and potentially improve synchronization to the environment and its (seasonal) changes. This is in line with the observation that VWR improves entrainment to a new circadian phase of the light-dark cycle in mice [62].

A recent review and consensus statement about travel fatigue and jet lag in athletes concluded that there is a lack of evidence for interventions that reduce the symptoms of jet lag, but exercise was identified as a potential strategy [63]. Our findings support the hypothesis that exercise may be a potential strategy to facilitate adaptation to a new circadian phase and thus reduce jet lag symptoms in humans, although this hypothesis remains to be investigated in humans. Thus, VWR-mediated repression of ΔFOSB, and its subsequent effects on neuronal excitability, may act as a molecular mechanism in the SCN linking physical exercise to altered circadian input and output.

Many rodent studies focusing on drugs of abuse and their addictive effects have revealed an important role for the induction of ΔFOSB in medium spiny neurons of the nucleus accumbens, in particular in relation to dopamine signaling and specifically via the dopamine d1 receptor (DRD1) [20]. For a long time it was thought that *ΔFosB* mRNA is generated constitutively above a certain level of *FosB* mRNA expression [64]. However, it was recently demonstrated that dopamine signaling can synergize with the TGFβ superfamily receptor ALK4 to potentiate the production of ΔFOSB in medium spiny neurons of the nucleus accumbens through activation of the RNA-binding proteins PCBP1 and SMAD3 [65]. Thus, it is not unlikely that dopamine- and/or TGFβ-signaling is also involved in the modulation of SCN ΔFOSB, especially since many DRD1-expressing neurons are present in the SCN [66, 67]. Additional studies are necessary to determine if dopamine- and/or TGFβ-signaling are also involved in the VWR-mediated suppression of SCN ΔFOSB and which neuronal markers, aside from VIP, identify the SCN neuron subtypes where this suppression occurs.

Which other molecular mechanisms can modulate SCN ΔFOSB? In this study we first observed that ΔFOSB-positive cell numbers in the SCN fluctuated across the estrous cycle in female sedentary rats, with the highest levels during diestrus and the lowest levels during proestrus. The estrous cycle is characterized by distinct hormonal changes, with an estrogen and progesterone surge during proestrus, and a second, but smaller, progesterone surge during metestrus/diestrus [68–70]. Estrogen signaling regulates VWR behavior, as female rats show cyclic variations in running linked to the estrous cycle, with ovariectomy reducing VWR and estrogen replacement restoring VWR [71]. Interestingly, we observed high levels of SCN ΔFOSB during metestrus/diestrus, which seems to coincide with low estrogen levels and a progesterone peak. However, physiological progesterone levels did previously not affect SCN *Per1*-Luc rhythms [72]. Therefore, we hypothesized that either an indirect pathway, or signaling via estrogen receptors and/or other receptors in the SCN, mediated the cyclicity in SCN ΔFOSB. Thus, we used estrogen replacement

in an OVX background to mechanistically determine that estradiol replacement following ovariectomy lowers ΔFOSB-positive cell numbers in the SCN, indicating that the fluctuation of ΔFOSB-positive cell numbers during the estrous cycle are mediated, at least in part, by estradiol. Circulating estrogenic hormones, including estradiol, are well known to affect the circadian system in rodents [71, 73, 74], but it is not clear whether these are direct effects at the level of the SCN or indirect via afferent projections. Whereas no clear observable effects of estradiol treatment on SCN explants were observed [72], nor of the estrous cycle itself on the rhythmicity of the expression of several major clock genes [75], estradiol can directly alter neuronal activity in the SCN [76]. Indeed, estradiol increased firing frequency and excitatory synaptic transmission of neurons in ventromedial SCN explants of male rats, and this effect was abolished by the administration of an estradiol antagonist [77]. Moreover, although ΔFOSB was not assessed directly, cFOS levels in the SCN were reported to be lower following ovariectomy in rats, and estradiol treatment successfully restored SCN cFOS levels during the light phase [78–80]. Furthermore, the start of feminizing gender affirming hormone treatment (including estradiol) in transgender humans, is associated with a 20 min forward chronotype shift [81]. Because ΔFOSB inhibits production of cFOS in mouse striatal neurons or dentate gyrus granule cells [30, 55], and post-partum estradiol withdrawal increases ΔFOSB in mouse nucleus accumbens [82], ΔFOSB might thus mediate some of the effects of estradiol on SCN function. For example, it was recently demonstrated in the nucleus accumbens that low-estrogenic females respond to acute cocaine by opening neuronal chromatin enriched for the sites of ΔFOSB, whereas high-estrogenic females respond to cocaine by preferential chromatin closing, providing a mechanism for limiting cocaine-driven chromatin and synaptic plasticity [83]. Similar mechanistic interactions between estrogen and ΔFOSB might also modify the response of the SCN to physiological stimuli, which could potentially explain chronotype differences observed between human sexes [84].

SCN ΔFOSB was predominantly localized to the lateral part of the ventral core region, but not to dorsal shell region (Supplemental Figs. 1a,b). Current understanding of the functional differences between these subregions indicates that the SCN core region receives, integrates, and converts various kinds of entraining information, amongst others to the dorsal shell, whereas the SCN shell region generates circadian oscillations and sends these entrained signals to the rest of the brain [42, 85]. The ventral core region receives entraining information from several sources, including photic input via the retinohypothalamic tract (RHT), serotonin (5-HT) input from the raphe nuclei,

dopaminergic input from the ventral tegmental area, and neuropeptide y (NPY) input from the intergeniculate leaflet (IGL) [86]. As all these areas also express estrogen receptors [87], we also cannot exclude the possibility of an indirect estrogen or progesterone effect. An interesting observation in this regard is a study in golden hamsters by LeSauter & Silver [88] who identified a SCN subregion involved in the regulation of locomotor activity. The localization of this subnucleus, characterized by the expression of the calcium-binding protein Calbindin-D$_{28K}$ (CaBP), shows strong similarity to the ΔFOSB-expressing subnucleus in the lateral part of the core SCN. In earlier SCN transplantation experiments the strength of the restored rhythm correlated with the number of CaBP-positive cells in the graft. Unfortunately, in the rat SCN, CaBP expression does not show such a localized expression but is scattered throughout the SCN [89]. Additional studies are necessary to determine which brain regions and/or signaling cascades are involved in the effects of VWR on SCN ΔFOSB.

A key contribution of the present study involves our demonstration that ΔFOSB-positive cell numbers in the SCN are modulated by voluntary running in both male and female rats and by the estrous cycle via, at least in part, estradiol in female rats. These findings increase our mechanistic understanding of how physical exercise and the estrous cycle regulate ΔFOSB in the SCN and how physical exercise potentially sensitizes the SCN. Targeting the key components of these mechanisms may lead to interventions that can help optimize the circadian impact of physical exercise or counteract the effects of circadian dysregulation.

DATA ACCESSIBILITY STATEMENT

Data will be made available on reasonable request.

ADDITIONAL FILE

The additional file for this article can be found as follows:

- **Supplemental figures.** Supplemental Figures 1 to 3. DOI: https://doi.org/10.5334/jcr.257.s1

ETHICS AND CONSENT

All animal housing and experiments were performed in accordance with the European guidelines for laboratory animals (EU directive 2010\63\EU). Protocols for animal experiments were approved by the Dutch Central Committee for Animal Experiments (CCD; project proposals AVD11800202316744, AVD80100202216157 and AVD8010020172424) and the Agency for Animal Welfare (IvD) committee of the Netherlands Institute for Neuroscience (Royal Dutch Academy of Sciences) in compliance with the European guidelines and regulations for the care and use of laboratory animals (EU directive 2010\63\EU).

COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

Ayano Shiba: Investigation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing; Marene Hardonk: Investigation, Formal analysis, Data curation, Writing – review & editing; Ewout Foppen: Supervision, Investigation, Writing – review & editing; Tess Kool: Investigation, Writing – review & editing; Susanne la Fleur: Funding acquisition, Supervision, Writing – review & editing; Paul Lucassen: Supervision, Writing – review & editing; Chun-Xia Yi: Conceptualization, Supervision, Writing – review & editing; Dirk Jan Stenvers: Funding

AUTHOR AFFILIATIONS

Ayano Shiba orcid.org/0009-0008-3404-9159
Netherlands Institute for Neuroscience, Institute of the Royal Netherlands Academy of Arts and Sciences (KNAW), Meibergdreef 47, 1105BA Amsterdam, The Netherlands; Amsterdam UMC, University of Amsterdam, Laboratory of Endocrinology, Department of Laboratory Medicine, Meibergdreef 9, 1105AZ, Amsterdam, The Netherlands; Amsterdam Gastroenterology, Endocrinology and Metabolism (AGEM), Amsterdam, The Netherlands

Marene H. Hardonk orcid.org/0009-0009-1755-8346
Brain Plasticity group, Swammerdam Institute for Life Sciences, Faculty of Science, Science Park 904, 1098XH Amsterdam, The Netherlands

Ewout Foppen orcid.org/0000-0001-9831-5630
Netherlands Institute for Neuroscience, Institute of the Royal Netherlands Academy of Arts and Sciences (KNAW), Meibergdreef 47, 1105BA Amsterdam, The Netherlands; Amsterdam UMC, University of Amsterdam, Laboratory of Endocrinology, Department of Laboratory Medicine, Meibergdreef 9, 1105AZ, Amsterdam, The Netherlands; Amsterdam Gastroenterology, Endocrinology and Metabolism (AGEM), Amsterdam, The Netherlands

Tess Kool
Netherlands Institute for Neuroscience, Institute of the Royal Netherlands Academy of Arts and Sciences (KNAW), Meibergdreef 47, 1105BA Amsterdam, The Netherlands; Amsterdam UMC, University of Amsterdam, Laboratory of Endocrinology, Department of Laboratory Medicine, Meibergdreef 9, 1105AZ, Amsterdam, The Netherlands; Amsterdam Gastroenterology, Endocrinology and Metabolism (AGEM), Amsterdam, The Netherlands

Susanne E. la Fleur orcid.org/0000-0002-4298-7451
Netherlands Institute for Neuroscience, Institute of the Royal Netherlands Academy of Arts and Sciences (KNAW), Meibergdreef 47, 1105BA Amsterdam, The Netherlands; Amsterdam UMC, University of Amsterdam, Laboratory of Endocrinology, Department of Laboratory Medicine, Meibergdreef 9, 1105AZ, Amsterdam, The Netherlands; Amsterdam Gastroenterology, Endocrinology and Metabolism (AGEM), Amsterdam, The Netherlands

Paul J. Lucassen orcid.org/0000-0001-9708-9133
Brain Plasticity group, Swammerdam Institute for Life Sciences, Faculty of Science, Science Park 904, 1098XH Amsterdam, The Netherlands; Centre for Urban Mental Health, University of Amsterdam, Amsterdam, The Netherlands

Chun-Xia Yi orcid.org/0000-0003-1184-4615
Netherlands Institute for Neuroscience, Institute of the Royal Netherlands Academy of Arts and Sciences (KNAW), Meibergdreef 47, 1105BA Amsterdam, The Netherlands; Amsterdam UMC,

University of Amsterdam, Laboratory of Endocrinology, Department of Laboratory Medicine, Meibergdreef 9, 1105AZ, Amsterdam, The Netherlands; Amsterdam Gastroenterology, Endocrinology and Metabolism (AGEM), Amsterdam, The Netherlands; Department of Endocrinology and Metabolism, Amsterdam UMC, University of Amsterdam, Meibergdreef 9, 1105AZ, Amsterdam, The Netherlands

Dirk Jan Stenvers orcid.org/0000-0002-9519-5140
Amsterdam Gastroenterology, Endocrinology and Metabolism (AGEM), Amsterdam, The Netherlands; Department of Endocrinology and Metabolism, Amsterdam UMC, University of Amsterdam, Meibergdreef 9, 1105AZ, Amsterdam, The Netherlands

Joram D. Mul orcid.org/0000-0003-0453-2568
Brain Plasticity group, Swammerdam Institute for Life Sciences, Faculty of Science, Science Park 904, 1098XH Amsterdam, The Netherlands; Centre for Urban Mental Health, University of Amsterdam, Amsterdam, The Netherlands

Andries Kalsbeek orcid.org/0000-0001-9606-8453
Netherlands Institute for Neuroscience, Institute of the Royal Netherlands Academy of Arts and Sciences (KNAW), Meibergdreef 47, 1105BA Amsterdam, The Netherlands; Amsterdam UMC, University of Amsterdam, Laboratory of Endocrinology, Department of Laboratory Medicine, Meibergdreef 9, 1105AZ, Amsterdam, The Netherlands; Amsterdam Gastroenterology, Endocrinology and Metabolism (AGEM), Amsterdam, The Netherlands; Department of Endocrinology and Metabolism, Amsterdam UMC, University of Amsterdam, Meibergdreef 9, 1105AZ, Amsterdam, The Netherlands

REFERENCES

1. **Hughes ATL, Piggins HD.** Feedback actions of locomotor activity to the circadian clock. *Prog Brain Res.* 2012;199:305–36. DOI: https://doi.org/10.1016/B978-0-444-59427-3.00018-6

2. **Mrosovsky N.** Beyond the suprachiasmatic nucleus. *Chronobiol Int.* 2003;20(1):1–8. DOI: https://doi.org/10.1081/CBI-120017811

3. **Arida RM, Teixeira-Machado L.** The Contribution of Physical Exercise to Brain Resilience. *Front Behav Neurosci.* 2020;14:626769. DOI: https://doi.org/10.3389/fnbeh.2020.626769

4. **Dishman RK, Berthoud HR, Booth FW, Cotman CW, Edgerton VR, Fleshner MR,** et al. Neurobiology of exercise. *Obes Silver Spring Md.* 2006 Mar;14(3):345–56. DOI: https://doi.org/10.1038/oby.2006.46

5. **Farmer J, Zhao X, van Praag H, Wodtke K, Gage FH, Christie BR.** Effects of voluntary exercise on synaptic plasticity and gene expression in the dentate gyrus of adult male Sprague-Dawley rats in vivo. *Neuroscience.* 2004;124(1):71–9. DOI: https://doi.org/10.1016/j.neuroscience.2003.09.029

6. **Gao Y, Syed M, Zhao X.** Mechanisms underlying the effect of voluntary running on adult hippocampal neurogenesis. *Hippocampus.* 2023 Apr;33(4):373–90. DOI: https://doi.org/10.1002/hipo.23520

7. **Greenwood BN, Foley TE, Le TV, Strong PV, Loughridge AB, Day HEW,** et al. Long-term voluntary wheel running is rewarding and produces plasticity in the mesolimbic reward pathway. *Behav Brain Res.* 2011 Mar 1;217(2):354–62. DOI: https://doi.org/10.1016/j.bbr.2010.11.005

8. **Mul JD.** Voluntary exercise and depression-like behavior in rodents: are we running in the right direction? *J Mol Endocrinol.* 2018 Apr;60(3):R77–95. DOI: https://doi.org/10.1530/JME-17-0165

9. **Deboer T, Tobler I.** Running wheel size influences circadian rhythm period and its phase shift in mice. *J Comp Physiol [A].* 2000 Oct;186(10):969–73. DOI: https://doi.org/10.1007/s003590000150

10. **Edgar DM, Martin CE, Dement WC.** Activity feedback to the mammalian circadian pacemaker: influence on observed measures of rhythm period length. *J Biol Rhythms.* 1991;6(3):185–99. DOI: https://doi.org/10.1177/074873049100600301

11. **Yamada N, Shimoda K, Ohi K, Takahashi S, Takahashi K.** Free-access to a running wheel shortens the period of free-running rhythm in blinded rats. *Physiol Behav.* 1988;42(1):87–91. DOI: https://doi.org/10.1016/0031-9384(88)90265-X

12. **Van Oosterhout F, Lucassen EA, Houben T, vanderLeest HT, Antle MC, Meijer JH.** Amplitude of the SCN Clock Enhanced by the Behavioral Activity Rhythm. In: Yamazaki S, editor. *PLoS ONE.* 2012 Jun 28;7(6):e39693. DOI: https://doi.org/10.1371/journal.pone.0039693

13. **Pendergast JS, Branecky KL, Huang R, Niswender KD, Yamazaki S.** Wheel-running activity modulates circadian organization and the daily rhythm of eating behavior. *Front Psychol.* 2014;5:177. DOI: https://doi.org/10.3389/fpsyg.2014.00177

14. **Schroeder AM, Truong D, Loh DH, Jordan MC, Roos KP, Colwell CS.** Voluntary scheduled exercise alters diurnal rhythms of behaviour, physiology and gene expression in wild-type and vasoactive intestinal peptide-deficient mice. *J Physiol.* 2012 Dec;590(23):6213–26. DOI: https://doi.org/10.1113/jphysiol.2012.233676

15. **Nestler EJ.** Transcriptional mechanisms of addiction: role of ΔFosB. *Philos Trans R Soc B Biol Sci.* 2008 Oct 12;363(1507):3245–55. DOI: https://doi.org/10.1098/rstb.2008.0067

16. **Nestler EJ.** Cellular basis of memory for addiction. *Dialogues Clin Neurosci.* 2013 Dec;15(4):431–43. DOI: https://doi.org/10.31887/DCNS.2013.15.4/enestler

17. **Nestler EJ.** ΔFosB: a transcriptional regulator of stress and antidepressant responses. *Eur J Pharmacol.* 2015 Apr 15;753:66–72. DOI: https://doi.org/10.1016/j.ejphar.2014.10.034

18. **Nestler EJ, Barrot M, Self DW.** ΔFosB: A sustained molecular switch for addiction. *Proc Natl Acad Sci U S A.* 2001 Sep 25;98(20):11042–6. DOI: https://doi.org/10.1073/pnas.191352698

19. **Robison AJ, Nestler EJ.** Transcriptional and epigenetic mechanisms of addiction. *Nat Rev Neurosci.* 2011 Oct 12;12(11):623–37. DOI: https://doi.org/10.1038/nrn3111

20. **Robison AJ, Nestler EJ.** ΔFOSB: A Potentially Druggable Master Orchestrator of Activity-Dependent Gene Expression. *ACS Chem Neurosci.* 2022 Feb 2;13(3):296–307. DOI: https://doi.org/10.1021/acschemneuro.1c00723

21. **Chen J, Kelz MB, Hope BT, Nakabeppu Y, Nestler EJ.** Chronic Fos-Related Antigens: Stable Variants of ΔFosB Induced in Brain by Chronic Treatments. *J Neurosci.* 1997 Jul 1. DOI: https://doi.org/10.1523/JNEUROSCI.17-13-04933.1997

22. **Hiroi N, Marek GJ, Brown JR, Ye H, Saudou F, Vaidya VA,** et al. Essential Role of the *fos* B Gene in Molecular, Cellular, and Behavioral Actions of Chronic Electroconvulsive Seizures. *J Neurosci.* 1998 Sep 1;18(17):6952–62. DOI: https://doi.org/10.1523/JNEUROSCI.18-17-06952.1998

23. **Yin Z, Venkannagari H, Lynch H, Aglyamova G, Bhandari M, Machius M,** et al. Self-assembly of the bZIP transcription factor ΔFosB. *Curr Res Struct Biol.* 2020;2:1–13. DOI: https://doi.org/10.1016/j.crstbi.2019.12.001

24. **Andersson M, Westin JE, Cenci MA.** Time course of striatal ΔFosB-like immunoreactivity and prodynorphin mRNA levels after discontinuation of chronic dopaminomimetic treatment. *Eur J Neurosci.* 2003 Feb;17(3):661–6. DOI: https://doi.org/10.1046/j.1460-9568.2003.02469.x

25. **Carle TL, Ohnishi YN, Ohnishi YH, Alibhai IN, Wilkinson MB, Kumar A,** et al. Proteasome-dependent and -independent mechanisms for FosB destabilization: identification of FosB degron domains and implications for ΔFosB stability. *Eur J Neurosci.* 2007 May;25(10):3009–19. DOI: https://doi.org/10.1111/j.1460-9568.2007.05575.x

26. **Cates HM, Thibault M, Pfau M, Heller E, Eagle A, Gajewski P,** et al. Threonine 149 Phosphorylation Enhances ΔFosB Transcriptional Activity to Control Psychomotor Responses to Cocaine. *J Neurosci.* 2014 Aug 20;34(34):11461–9. DOI: https://doi.org/10.1523/JNEUROSCI.1611-14.2014

27. **Hope BT, Nye HE, Kelz MB, Self DW, Iadarola MJ, Nakabeppu Y,** et al. Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. *Neuron.* 1994 Nov;13(5):1235–44. DOI: https://doi.org/10.1016/0896-6273(94)90061-2

28. **Kreuter JD, Mattson BJ, Wang B, You ZB, Hope BT.** Cocaine-induced Fos expression in rat striatum is blocked by chloral hydrate or urethane. *Neuroscience.* 2004 Jan;127(1):233–42. DOI: https://doi.org/10.1016/j.neuroscience.2004.04.047

29. **Ulery-Reynolds PG, Castillo MA, Vialou V, Russo SJ, Nestler EJ.** Phosphorylation of ΔFosB mediates its stability in vivo. *Neuroscience.* 2009 Jan;158(2):369–72. DOI: https://doi.org/10.1016/j.neuroscience.2008.10.059

30. **Lamothe-Molina PJ, Franzelin A, Beck L, Li D, Auksutat L, Fieblinger T,** et al. ΔFosB accumulation in hippocampal granule cells drives cFos pattern separation during spatial learning. *Nat Commun.* 2022 Oct 26;13:6376. DOI: https://doi.org/10.1038/s41467-022-33947-w

31. **McClung CA, Ulery PG, Perrotti LI, Zachariou V, Berton O, Nestler EJ.** ΔFosB: a molecular switch for long-term adaptation in the brain. *Mol Brain Res.* 2004 Dec;132(2):146–54. DOI: https://doi.org/10.1016/j.molbrainres.2004.05.014

32. **Patterson JR, Kim EJ, Goudreau JL, Lookingland KJ.** FosB and ΔFosB expression in brain regions containing differentially susceptible dopamine neurons following acute neurotoxicant exposure. *Brain Res.* 2016 Oct;1649:53–66. DOI: https://doi.org/10.1016/j.brainres.2016.08.030

33. **Ebling FJP, Maywood ES, Mehta M, Hancock DC, McNULTY S, De Bono J,** et al. FosB in the Suprachiasmatic Nucleus of the Syrian and Siberian Hamster. *Brain Res Bull.* 1996;41(5):257–68. DOI: https://doi.org/10.1016/S0361-9230(96)00192-X

34. **Mikkelsen JD, Vrang N, Mrosovsky N.** Expression of Fos in the circadian system following nonphotic stimulation. *Brain Res Bull.* 1998 Nov;47(4):367–76. DOI: https://doi.org/10.1016/S0361-9230(98)00121-X

35. **Peters RV, Aronin N, Schwartz WJ.** Circadian regulation of Fos B is different from c-Fos in the rat suprachiasmatic nucleus. *Mol Brain Res.* 1994 Dec;27(2):243–8. DOI: https://doi.org/10.1016/0169-328X(94)90006-X

36. **Schwartz WJ, Carpino A, de la Iglesia HO, Baler R, Klein DC, Nakabeppu Y,** et al. Differential regulation of fos family genes in the ventrolateral and dorsomedial subdivisions of the rat suprachiasmatic nucleus. *Neuroscience.* 2000;98(3):535–47. DOI: https://doi.org/10.1016/S0306-4522(00)00140-8

37. **Takeuchi J, Shannon W, Aronin N, Schwartz WJ.** Compositional changes of AP-1 DNA-binding proteins are regulated by light in a mammalian circadian clock. *Neuron.* 1993 Nov;11(5):825–36. DOI: https://doi.org/10.1016/0896-6273(93)90112-5

38. **Perrotti LI, Hadeishi Y, Ulery PG, Barrot M, Monteggia L, Duman RS,** et al. Induction of ΔFosB in Reward-Related Brain Structures after Chronic Stress. *J Neurosci.* 2004 Nov 24;24(47):10594–602. DOI: https://doi.org/10.1523/JNEUROSCI.2542-04.2004

39. **Gordon MN, Osterburg HH, May PC, Finch CE.** Effective oral administration of 17 beta-estradiol to female C57BL/6J mice through the drinking water. *Biol Reprod.* 1986 Dec;35(5):1088–95. DOI: https://doi.org/10.1095/biolreprod35.5.1088

40. **Lowry NC, Pardon LP, Yates MA, Juraska JM.** Effects of long term treatment with 17 β-estradiol and medroxyprogesterone acetate on water maze performance in middle aged female rats. *Horm Behav.* 2010 Jul;58(2):200–7. DOI: https://doi.org/10.1016/j.yhbeh.2010.03.018

41. **Bankhead P, Loughrey MB, Fernández JA, Dombrowski Y, McArt DG, Dunne PD,** et al. QuPath: Open source software for digital pathology image analysis. *Sci Rep.* 2017 Dec 4;7(1):16878. DOI: https://doi.org/10.1038/s41598-017-17204-5

42. **Colwell CS.** Linking neural activity and molecular oscillations in the SCN. *Nat Rev Neurosci.* 2011 Sep 2;12(10):553–69. DOI: https://doi.org/10.1038/nrn3086

43. **Arnold MR, Greenwood BN, McArthur JA, Clark PJ, Fleshner M, Lowry CA.** Effects of repeated voluntary or forced exercise on brainstem serotonergic systems in rats. *Behav Brain Res.* 2020 Jan 27;378:112237. DOI: https://doi.org/10.1016/j.bbr.2019.112237

44. **Carhuatanta KA, Demuro G, Tschöp MH, Pfluger PT, Benoit SC, Obici S.** Voluntary exercise improves high-fat diet-induced leptin resistance independent of adiposity. *Endocrinology.* 2011 Jul;152(7):2655–64. DOI: https://doi.org/10.1210/en.2010-1340

45. **Greenwood BN, Foley TE, Le TV, Strong PV, Loughridge AB, Day HEW,** et al. Long-term voluntary wheel running is rewarding and produces plasticity in the mesolimbic reward pathway. *Behav Brain Res.* 2011 Mar 1;217(2):354–62. DOI: https://doi.org/10.1016/j.bbr.2010.11.005

46. **Herrera JJ, Fedynska S, Ghasem PR, Wieman T, Clark PJ, Gray N,** et al. Neurochemical and behavioral indices of exercise reward are independent of exercise controllability. *Eur J Neurosci.* 2016 May;43(9):1190–202. DOI: https://doi.org/10.1111/ejn.13193

47. **Mul JD, Soto M, Cahill ME, Ryan RE, Takahashi H, So K,** et al. Voluntary wheel running promotes resilience to chronic social defeat stress in mice: a role for nucleus accumbens ΔFosB. *Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol.* 2018 Aug;43(9):1934–42. DOI: https://doi.org/10.1038/s41386-018-0103-z

48. **Nishijima T, Kawakami M, Kita I.** Long-Term Exercise Is a Potent Trigger for ΔFosB Induction in the Hippocampus along the dorso–ventral Axis. *PLOS ONE.* 2013 Nov 25;8(11):e81245. DOI: https://doi.org/10.1371/journal.pone.0081245

49. **Obici S, Magrisso IJ, Ghazarian AS, Shirazian A, Miller JR, Loyd CM,** et al. Moderate voluntary exercise attenuates the metabolic syndrome in melanocortin-4 receptor-deficient rats showing central dopaminergic dysregulation. *Mol Metab.* 2015 Jul 17;4(10):692–705. DOI: https://doi.org/10.1016/j.molmet.2015.07.003

50. **Triviño-Paredes J, Patten AR, Gil-Mohapel J, Christie BR.** The effects of hormones and physical exercise on hippocampal structural plasticity. *Front Neuroendocrinol.*

2016 Apr;41:23–43. DOI: https://doi.org/10.1016/j.yfrne.2016.03.001

51. **Watanasriyakul WT, Normann MC, Akinbo OI, Colburn W, Dagner A, Grippo AJ.** Protective neuroendocrine effects of environmental enrichment and voluntary exercise against social isolation: evidence for mediation by limbic structures. *Stress.* 2019 Sep 3;22(5):603–18. DOI: https://doi.org/10.1080/10253890.2019.1617691

52. **Werme M, Messer C, Olson L, Gilden L, Thorén P, Nestler EJ,** et al. ΔFosB Regulates Wheel Running. *J Neurosci.* 2002 Sep 15;22(18):8133–8. DOI: https://doi.org/10.1523/JNEUROSCI.22-18-08133.2002

53. **McClung CA, Nestler EJ.** Regulation of gene expression and cocaine reward by CREB and DeltaFosB. *Nat Neurosci.* 2003 Nov;6(11):1208–15. DOI: https://doi.org/10.1038/nn1143

54. **Corbett BF, You JC, Zhang X, Pyfer MS, Tosi U, Iascone DM,** et al. ΔFosB Regulates Gene Expression and Cognitive Dysfunction in a Mouse Model of Alzheimer's Disease. *Cell Rep.* 2017 Jul 11;20(2):344–55. DOI: https://doi.org/10.1016/j.celrep.2017.06.040

55. **Renthal W, Carle TL, Maze I, Covington HE, Truong HT, Alibhai I,** et al. ΔFosB Mediates Epigenetic Desensitization of the c-fos Gene After Chronic Amphetamine Exposure. *J Neurosci.* 2008 Jul 16;28(29):7344–9. DOI: https://doi.org/10.1523/JNEUROSCI.1043-08.2008

56. **Eagle AL, Williams ES, Beatty JA, Cox CL, Robison AJ.** ΔFosB Decreases Excitability of Dorsal Hippocampal CA1 Neurons. *eNeuro.* 2018 Aug 3;5(4):ENEURO.0104-18.2018. DOI: https://doi.org/10.1523/ENEURO.0104-18.2018

57. **Chen J, Zhang Y, Kelz MB, Steffen C, Ang ES, Zeng L,** et al. Induction of cyclin-dependent kinase 5 in the hippocampus by chronic electroconvulsive seizures: role of [Delta]FosB. *J Neurosci Off J Soc Neurosci.* 2000 Dec 15;20(24):8965–71. DOI: https://doi.org/10.1523/JNEUROSCI.20-24-08965.2000

58. **Kelz MB, Chen J, Carlezon WA, Whisler K, Gilden L, Beckmann AM,** et al. Expression of the transcription factor deltaFosB in the brain controls sensitivity to cocaine. *Nature.* 1999 Sep 16;401(6750):272–6. DOI: https://doi.org/10.1038/45790

59. **Robison AJ, Vialou V, Mazei-Robison M, Feng J, Kourrich S, Collins M,** et al. Behavioral and Structural Responses to Chronic Cocaine Require a Feedforward Loop Involving ΔFosB and Calcium/Calmodulin-Dependent Protein Kinase II in the Nucleus Accumbens Shell. *J Neurosci.* 2013 Mar 6;33(10):4295–307. DOI: https://doi.org/10.1523/JNEUROSCI.5192-12.2013

60. **Robison AJ, Vialou V, Sun HS, Labonte B, A Golden S, Dias C,** et al. Fluoxetine Epigenetically Alters the CaMKIIα Promoter in Nucleus Accumbens to Regulate ΔFosB Binding and Antidepressant Effects. *Neuropsychopharmacology.* 2014 Apr;39(5):1178–86. DOI: https://doi.org/10.1038/npp.2013.319

61. **Vialou V, Robison AJ, LaPlant QC, Covington HE, Dietz DM, Ohnishi YN,** et al. ΔFosB in brain reward circuits mediates resilience to stress and antidepressant responses. *Nat Neurosci.* 2010 Jun;13(6):745–52. DOI: https://doi.org/10.1038/nn.2551

62. **Oneda S, Cao S, Haraguchi A, Sasaki H, Shibata S.** Wheel-Running Facilitates Phase Advances in Locomotor and Peripheral Circadian Rhythm in Social Jet Lag Model Mice. *Front Physiol.* 2022;13:821199. Published 2022 Feb 16. DOI: https://doi.org/10.3389/fphys.2022.821199

63. **Janse van Rensburg DC, Jansen van Rensburg A, Fowler PM, Bender AM, Stevens D, Sullivan KO,** et al. Managing Travel Fatigue and Jet Lag in Athletes: A Review and Consensus Statement. *Sports Med.* 2021 Oct 1;51(10):2029–50. DOI: https://doi.org/10.1007/s40279-021-01502-0

64. **Alibhai IN, Green TA, Potashkin JA, Nestler EJ.** Regulation of fosB and DeltafosB mRNA expression: in vivo and in vitro studies. *Brain Res.* 2007 Apr 27;1143:22–33. DOI: https://doi.org/10.1016/j.brainres.2007.01.069

65. **Krapacher FA, Fernández-Suárez D, Andersson A, Carrier-Ruiz A, Ibáñez CF.** Convergent dopamine and ALK4 signaling to PCBP1 controls FosB alternative splicing and cocaine behavioral sensitization. *EMBO J.* 2022 Aug;41(15):e110721. DOI: https://doi.org/10.15252/embj.2022110721

66. **Grippo RM, Purohit AM, Zhang Q, Zweifel LS, Güler AD.** Direct Midbrain Dopamine Input to the Suprachiasmatic Nucleus Accelerates Circadian Entrainment. *Curr Biol.* 2017 Aug;27(16):2465–2475.e3. DOI: https://doi.org/10.1016/j.cub.2017.06.084

67. **Jones JR, Tackenberg MC, McMahon DG.** Manipulating circadian clock neuron firing rate resets molecular circadian rhythms and behavior. *Nat Neurosci.* 2015 Mar;18(3):373–5. DOI: https://doi.org/10.1038/nn.3937

68. **Scharfman HE, Mercurio TC, Goodman JH, Wilson MA, MacLusky NJ.** Hippocampal Excitability Increases during the Estrous Cycle in the Rat: A Potential Role for Brain-Derived Neurotrophic Factor. *J Neurosci.* 2003 Dec 17;23(37):11641–52. DOI: https://doi.org/10.1523/JNEUROSCI.23-37-11641.2003

69. **Smith MS, Freeman ME, Neill JD.** The Control of Progesterone Secretion During the Estrous Cycle and Early Pseudopregnancy in the Rat: Prolactin, Gonadotropin and Steroid Levels Associated with Rescue of the Corpus Luteum of Pseudopregnancy. *Endocrinology.* 1975 Jan;96(1):219–26. DOI: https://doi.org/10.1210/endo-96-1-219

70. **Walmer DK, Wrona MA, Hughes CL, Nelson KG.** Lactoferrin expression in the mouse reproductive tract during the natural estrous cycle: correlation with circulating estradiol and progesterone. *Endocrinology.* 1992 Sep;131(3):1458–66. DOI: https://doi.org/10.1210/endo.131.3.1505477

71. **Mathis V, Wegman-Points L, Pope B, Lee CMJ, Mohamed M, Rhodes JS,** et al. Estrogen-mediated individual differences

in female rat voluntary running behavior. *J Appl Physiol.* 2024 Mar;136(3):592–605. DOI: https://doi.org/10.1152/japplphysiol.00611.2023

72. **Murphy ZC, Pezuk P, Menaker M, Sellix MT.** Effects of Ovarian Hormones on Internal Circadian Organization in Rats. *Biol Reprod.* 2013 Aug;89(2):35. DOI: https://doi.org/10.1095/biolreprod.113.109322

73. **Morin LP, Fitzgerald KM, Zucker I.** Estradiol shortens the period of hamster circadian rhythms. *Science.* 1977 Apr 15;196(4287):305–7. DOI: https://doi.org/10.1126/science.557840

74. **Albers HE, Gerall AA, Axelson JF.** Effect of reproductive state on circadian periodicity in the rat. *Physiol Behav.* 1981 Jan;26(1):21–5. DOI: https://doi.org/10.1016/0031-9384(81)90073-1

75. **Perrin JS, Segall LA, Harbour VL, Woodside B, Amir S.** The expression of the clock protein PER2 in the limbic forebrain is modulated by the estrous cycle. *Proc Natl Acad Sci U S A.* 2006 Apr 4;103(14):5591–6. DOI: https://doi.org/10.1073/pnas.0601310103

76. **Krajnak K, Kashon ML, Rosewell KL, Wise PM.** Sex differences in the daily rhythm of vasoactive intestinal polypeptide but not arginine vasopressin messenger ribonucleic acid in the suprachiasmatic nuclei. *Endocrinology.* 1998 Oct;139(10):4189–96. DOI: https://doi.org/10.1210/en.139.10.4189

77. **Fatehi M, Fatehi-Hassanabad Z.** Effects of 17beta-estradiol on neuronal cell excitability and neurotransmission in the suprachiasmatic nucleus of rat. *Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol.* 2008 May;33(6):1354–64. DOI: https://doi.org/10.1038/sj.npp.1301523

78. **Alvord V, Kantra E, Pendergast JS.** Estrogens and the Circadian System. *Semin Cell Dev Biol.* 2022 Jun;126:56–65. DOI: https://doi.org/10.1016/j.semcdb.2021.04.010

79. **Peterfi Z, Churchill L, Hajdu I, Jr FO, Krueger JM, Parducz A.** Fos-immunoreactivity in the hypothalamus: dependency on the diurnal rhythm, sleep, gender, and estrogen. *Neuroscience.* 2004 Jan 1;124(3):695–707. DOI: https://doi.org/10.1016/j.neuroscience.2003.10.047

80. **Takamata A, Torii K, Miyake K, Morimoto K.** Chronic oestrogen replacement in ovariectomised rats attenuates food intake and augments c-Fos expression in the suprachiasmatic nucleus specifically during the light phase. *Br J Nutr.* 2011 Oct;106(8):1283–9. DOI: https://doi.org/10.1017/S0007114511001607

81. **Morssinkhof MWL, Zwager A, van der Tuuk K, den Heijer M, van der Werf YD, Stenvers DJ,** et al. Chronotype changes after sex hormone use: A prospective cohort study in transgender users of gender-affirming hormones. *Chronobiol Int.* 2024 May 3;41(5):658–68. DOI: https://doi.org/10.1080/07420528.2024.2339989

82. **Foster WB, Beach KF, Carson PF, Harris KC, Alonso BL, Costa LT,** et al. Estradiol withdrawal following a hormone simulated pregnancy induces deficits in affective behaviors and increases ΔFosB in D1 and D2 neurons in the nucleus accumbens core in mice. *Horm Behav.* 2023 Mar 1;149:105312. DOI: https://doi.org/10.1016/j.yhbeh.2023.105312

83. **Rocks D, Jaric I, Bellia F, Cham H, Greally JM, Suzuki M,** et al. Early-life stress and ovarian hormones alter transcriptional regulation in the nucleus accumbens resulting in sex-specific responses to cocaine. *Cell Rep.* 2023 Oct 31;42(10):113187. DOI: https://doi.org/10.1016/j.celrep.2023.113187

84. **Roenneberg T, Kuehnle T, Pramstaller PP, Ricken J, Havel M, Guth A,** et al. A marker for the end of adolescence. *Curr Biol CB.* 2004 Dec 29;14(24):R1038–1039. DOI: https://doi.org/10.1016/j.cub.2004.11.039

85. **Li Y, Androulakis IP.** Light entrainment of the SCN circadian clock and implications for personalized alterations of corticosterone rhythms in shift work and jet lag. *Sci Rep.* 2021 Sep 9;11(1):17929. DOI: https://doi.org/10.1038/s41598-021-97019-7

86. **Yan L, Karatsoreos I, LeSauter J, Welsh DK, Kay S, Foley D,** et al. Exploring Spatiotemporal Organization of SCN Circuits. *Cold Spring Harb Symp Quant Biol.* 2007;72:527–41. DOI: https://doi.org/10.1101/sqb.2007.72.037

87. **Bailey M, Silver R.** Sex differences in circadian timing systems: implications for disease. *Front Neuroendocrinol.* 2014 Jan;35(1):111–39. DOI: https://doi.org/10.1016/j.yfrne.2013.11.003

88. **LeSauter J, Silver R.** Localization of a suprachiasmatic nucleus subregion regulating locomotor rhythmicity. *J Neurosci Off J Soc Neurosci.* 1999 Jul 1;19(13):5574–85. DOI: https://doi.org/10.1523/JNEUROSCI.19-13-05574.1999

89. **Beaulé C, Amir S.** Effect of 192 IgG-saporin on circadian activity rhythms, expression of P75 neurotrophin receptors, calbindin-D28K, and light-induced Fos in the suprachiasmatic nucleus in rats. *Exp Neurol.* 2002 Aug;176(2):377–89. DOI: https://doi.org/10.1006/exnr.2002.7969

]u[&