### Title

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# Photoperiod-induced neurotransmitter plasticity declines with aging: An epigenetic regulation?

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## Abstract

Neuroplasticity has classically been understood to arise through changes in synaptic strength or synaptic connectivity. A newly discovered form of neuroplasticity, neurotransmitter switching, involves changes in neurotransmitter identity. Chronic exposure to different photoperiods alters the number of dopamine (tyrosine hydroxylase, TH+) and somatostatin (SST+) neurons in the paraventricular nucleus (PaVN) of the hypothalamus of adult rats and results in discrete behavioral changes. Here, we investigate whether photoperiod-induced neurotransmitter switching persists during aging and whether epigenetic mechanisms of histone acetylation and DNA methylation may contribute to this neurotransmitter plasticity. We show that this plasticity in rats is robust at 1 and at 3 months but reduced in TH+ neurons at 12 months and completely abolished in both TH+ and SST+ neurons by 18 months. De novo expression of DNMT3a catalyzing DNA methylation and anti-AcetylH3 assessing histone 3 acetylation were observed following short-day photoperiod exposure in both TH+ and SST+ neurons at 1 and 3 months while an overall increase in DNMT3a in SST+ neurons paralleled neuroplasticity reduction at 12 and 18 months. Histone acetylation increased in TH+ neurons and decreased in SST+ neurons following short-day exposure at 3 months while the total number of anti-AcetylH3+ PaVN neurons remained constant. Reciprocal histone acetylation in TH+ and SST+ neurons indicates the importance of studying epigenetic regulation at the circuit level for identified cell phenotypes. The findings may be useful for developing approaches for noninvasive treatment of disorders characterized by neurotransmitter dysfunction.

**KEYWORDS**

DNA methylation, dopamine, histone acetylation, hypothalamus, somatotostatin, RRID: RGD_5508398, RRID:AB_1977483, RRID:AB_2195927, RRID:AB_789607, RRID: AB_2115283, RRID:AB_2556546, RRID:AB_2715537, RRID:AB_162542

## 1 | INTRODUCTION

During embryonic development, genetic programs organize expression patterns of neurotransmitters within specific neuronal networks and activity-dependent mechanisms mediated by calcium signaling

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enable refinement of neurotransmitter expression (Borodinsky et al., 2004; Rosenberg & Spitzer, 2011). Experience-dependent neurotransmitter plasticity extends beyond this early phase. Induced by natural stimuli during development and in the mature nervous system, it entails addition, loss, or switching of neurotransmitters in a process known as neurotransmitter respecification (Dulcis et al., 2017; Dulcis, Jamshidi, Leutgeb, & Spitzer, 2013; Dulcis & Spitzer, 2008; Spitzer, 2015).

Seasonal changes in photoperiod act as environmental stressors, affecting the expression of clock genes that control functions of the circadian pacemaker, the suprachiasmatic nucleus (SCN) of the hypothalamus. SCN neurons make connections to the paraventricular nucleus (PaVN) of the hypothalamus that integrates neuroendocrine and autonomic functions (Ferguson, Latchford, & Samson, 2008). Local PaVN interneurons expressing TH or SST or both make synapses on corticotropin-releasing factor (CRF) cells (Cummings, Elde, Ells, & Lindall, 1983; Dulcis et al., 2013; Kumar, 2007), modulating release of CRF and regulating stress responses (Herman & Rosenmund, 2015). Exposure of adult rats to long-day (19 L:5D) or short-day (5L:19D) photoperiods leads to a shift in the number of dopaminergic (DA + TH+) and somatostatin (SST+) neurons in the PaVN, resulting in behavioral changes associated with stress responses (Dulcis et al., 2013). Rats exposed to long-day photoperiods for 1 week exhibit an increase in SST+ neurons and a decrease in TH+ neurons that cause more depressive- and anxiety-like behaviors when tested on the elevated plus maze and forced swim tests and display a higher titer of stress hormone CRF in their cerebral spinal fluid. Short-day exposure causes the opposite effects on the number of TH+ and SST+ neurons, stress responses, and CRH titer (Dulcis et al., 2013). Presynaptic changes in neurotransmitter identity were matched by changes in post-synaptic receptors in CRF cells, regulating stress responses. Neurotransmitter switching occurs through recruitment of "reserve pool" neurons that adapt circuit function by changing neurotransmitter identity (Dulcis & Spitzer, 2012).

The activity-dependent genetic regulation of photoperiod-induced neurotransmitter plasticity affecting behavior has implications for people who suffer from seasonal affective disorder (SAD) as well as major depressive disorders (Lam et al., 2016; Lam, Levitt, Levitan, et al., 2006). Due to its seasonal recurrence and equal incidence among men and women, SAD is a serious mental health problem (Kurlansik and Ibay, 2012). Because SAD's prevalence declines with age (Kurlansik & Ibay, 2012), it is of interest to investigate whether photoperiod-induced neurotransmitter plasticity is affected by aging.

We hypothesized that DNA modification has a role in photoperiod-induced plasticity, given that transmitter switching requires chronic neuronal activation (Meng, Li, Deisseroth, Leutgeb, & Spitzer, 2018) and that neuronal activity modifies chromatin accessibility and gene expression via epigenetic mechanisms in adult neurons (He et al., 2011; Su et al., 2017). While de novo transcriptional regulation has been shown to be the mechanism behind light-induced dopamine/SST transmitter switching (Dulcis et al., 2013), it was unknown whether DNA methylation or histone acetylation is potential environmental links initiating the genetic cascade regulating this form of plasticity. DNA methylation is essential for cellular mechanisms involved in synaptic plasticity, neuronal repair, neuronal survival, and learning and memory (Fan et al., 2001; Feng et al., 2010; Iskandar et al., 2010; Lardenoije et al., 2015). It is generally associated with gene repression (Moore, Le, & Fan, 2013). However, methylation of specific local nonpromoter sites can also result in gene activation and hypermethylation of certain genes results in increased expression (Silva et al., 2008; Wu et al., 2010). Histone acetylation makes DNA more accessible to regulatory proteins and transcription factors (Grunstein, 1997). The roles of de novo DNA methylation and histone acetylation in neural plasticity and emotional behavior (Oliveira, Hemstedt, & Bading, 2012) suggest that these processes could have particular relevance to the mechanism of neurotransmitter respecification in the mature and aging brain.

In this study, we provide evidence that neurotransmitter plasticity is age dependent and discover cell-specific patterns of expression of enzymes enabling DNA methylation and histone acetylation in response to changes in photoperiod.

## 2 | MATERIALS AND METHODS

### 2.1 | Animals

Male Long Evans rats (RRID:RGD_5508398, Harlan Industries, Indianapolis, IN) were separated into groups by age (1, 3, 12, and 18 months) and by experimental photoperiod conditions. Animals were exposed for 1 week to controlled light/dark cycles consisting of either a long day (19 hr light:5 hr dark [19 L:5D]), normal day (12 hr light:12 hr dark [12 L:12D]) or short day (5 hr light:19 hr dark [5 L:19D]). During photoperiod exposure, rats were singly housed with food and water ad libitum. All protocols were in strict adherence to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and approved by the IACUC at University of California, San Diego.

### 2.2 | Photoperiod chambers

Wooden photoperiod-chamber boxes were purchased from Actimetrics (Wilmette, IL). Each box was equipped with its own ventilation system, LED lights (134 LUX) set on timers to specific photoperiod conditions and completely sealed off from external room lighting. Animals were allowed to habituate in the chambers for 48 hr on a normal 12L:12D day. Following habituation, animals were exposed to their respective photoperiod conditions for 1 week, as 1 week has been shown to be sufficient time to induce neurotransmitter switching in the adult rat hypothalamus (Dulcis et al., 2013).

### 2.3 | Immunohistochemistry

Immediately after 1 week of exposure, all animals were perfused during the light phase of their light/dark cycle. The numbers of TH- and SST-immunoreactive cells do not change during the course of a diurnal cycle (Dulcis et al., 2013). Rats were anesthetized with 5% isoflurane (Vet One, Boise, ID), followed by an intraperitoneal (i.p.) injection of sodium pentobarbital (Vortech Pharmaceuticals, Dearborn, MI) at a dose of

150 mg/kg. Animals were then transcardially perfused with 1× PBS until perfusate was clear (250–300 mL), followed by ice-cold 4% paraformaldehyde (MP Biomedicals, Santa Ana, CA) until the neck and hindquarters were rigid (300 mL). Brains were extracted, post-fixed overnight in 4% paraformaldehyde, and then cryoprotected in 30% sucrose for 48 hr. Once cryoprotected, brains were flash frozen on dry ice and 30 μm-thick coronal sections were collected in a 1:12 series using a Leica SM2010R microtome. Tissue sections were stored in cryoprotectant (30% glycerol, 30% ethylene-glycol in 1xPBS, Fisher Bioreagents, Fair Lawn, NJ) at −20° C until ready for processing.

Immunohistochemistry for tyrosine hydroxylase (TH) and somatostatin (SST) was performed on free-floating sections using the standard avidin-biotin peroxidase method (Dulcis et al., 2013). Tissue sections were rinsed through a succession of 1× PBS washes, blocked for 30 min at room temperature with 5% normal horse serum (Vector Laboratories, Burlingame, CA) in 0.3% PBS-TX (Triton X-100, Fisher Bioreagents, Fair Lawn, NJ), and incubated in either mouse anti-TH (1:1000) (Millipore, Temecula, CA, RRID:AB_1977483) overnight or rabbit anti-SST (1:500) (Santa Cruz Biotechnology, Dallas, TX; RRID: AB_2195927) for 48 hr at 4°C. Tissue sections were then rinsed through another series of 1× PBS washes and incubated in either biotinylated anti-mouse IgG or anti-goat IgG secondary antibody (1:200) (Vector Laboratories, Burlingame, CA). The signal was amplified using a Vectastain ABC kit (Vector Laboratories) and developed with diaminobenzidine (DAB, Acros Organics, Morris Plains, NJ). Sections were mounted on superfrost slides (Fisher Scientific. Pittsburgh, PA) in 0.2% gelatin (Millipore, Burlington, MA), dried overnight at 21°C, dehydrated and coverslipped with Cytoseal (Thermoscientific, Kalamazoo, MI). Tissue sets stained for TH were counterstained using a Giemsa stain (1:2 solution in 0.1× PBS, Harleco, EMDmillipore, Temecula, CA) to identify the boundaries of the PaVN.

Triple-label immunofluorescence was used to identify expression of DNMT3a and AcetylH3 in TH+ and SST+ neurons of the paraventricular nucleus of the hypothalamus (PaVN). Free-floating tissue sections were rinsed with 1× PBS, blocked with 5% normal horse serum in 0.3% PBS-TX and labeled with either rabbit anti-DNMT3a (1:500) (Santa Cruz Biotechnology; RRID:AB_789607) or rabbit anti-AcetylH3 (1:500) (Millipore, Temecula, CA; RRID:AB_2115283), along with mouse anti-TH (1:1000) (Millipore) and goat anti-SST (1:500) (Santa Cruz Biotechnology) for 48 hr at 4°C. Tissue sections were then rinsed through another succession of 1× PBS washes and labeled with species-matched secondary antibody AlexaFluor conjugates (1:300): donkey anti-rabbit 488 (RRID: AB_2556546), donkey anti-goat 555 (RRID:AB_2715537), and donkey anti-mouse 647 (RRID:AB_162542) (Invitrogen, Carlsbad, CA). Sections were rinsed again in 1× PBS, mounted on superfrost slides in 0.2% gelatin and coverslipped with Fluoromount-G (Southern Biotech, Birmingham, AL).

## 2.4 | Imaging and neuronal quantification

Images of TH+ and SST+ DAB-labeled tissue were captured in bright field with either a Hamamatsu Nanozoomer 2.0HT slide scanner or a Leica DM4 B Stereologer for stereological quantification that is the current gold standard for these studies. Stereo Investigator software (MBF Bioscience) was used to count DAB immunostained cells. Counting was carried out using optical fractionator sampling on a Zeiss Axioskop 2 microscope (40×/0.65 Ph2 objective) equipped with a motorized stage. Pilot experiments with continuous counting determined that counting every other section was sufficient to estimate the number of TH+ and SST+ neurons in the PaVN of each brain. Fluorescence microscopy was performed on a Leica TCS DMi8 confocal microscope. System optimized z-stacks were collected and processed using the LAS AF software. Cell counts of epifluorescence immunoreactivity were performed using Adobe Photoshop CC.

## 2.5 | qPCR

Immediately after photoperiod exposure, rat brains were rapidly extracted and the hypothalamus was dissected in an RNAse-free environment and processed for qPCR. Bilateral tissue punches (1.5 mm) were collected from the PaVN and stored in RNAlater (ThermofFisher Scientific, Waltham, MA). Tissue punches were homogenized in Trizol (Thermofisher) using 0.5 mm zirconium oxide beads and a bullet blender (Next Advance Inc., Troy, NY). RNA was purified with Direct-zol kit (Zymo, Irvine, CA) and converted to cDNA using an iScrip kit (Biorad, Hercules, CA) following manufacturer's instructions. RT-PCR was performed in a Biorad CFX384 using KiCqStart SYBR Green Ready Mix (Millipore-Sigma, St. Louis, MO). RNA expression was normalized to the housekeeping gene GADPH and quantified using the ΔΔCt method. Primer pair (5′-3′) sequences used for each candidate gene are listed as follows:

*Dnmt1* (F: TTCTCGGCAGGGTATG; R: GTCTACCGACTGGGTGACA GT), *Dnmt3a* (F: ACGCCAAAGAAGTGTCTGCT; R: CTTTGCCCTGCTTT ATGGAG), *Dnmt3b* (F:CATAA GTCGAAGGTGCGTCGT; R: ACTTTTGTT CTCGCGTCTCCT), *G9a* (*F*: GACAACAAG GATGGTGAGGTC; *R*: AGCAT GAAGACCCGAACAG), *GCN5* (F: CATCGGTGGGATT TGCTT; R: GT ACTCGTCGGCGTAGGTG), *P300* (F: GGGACTAACCAATGGTGGTG; R: ATTGGGAGAAGTCAAGCCTG), *CBP* (F: GACCAAGATGGGGATGACTG; R: CCA CTGATGTTTGCAACTGG), Housekeeping gene *GAPDH* (F: TAT GATGACATCAAGG TGG; R: CACCACCCTGTTGCTGTA).

## 2.6 | Experimental design and statistical analysis

We hypothesized that the extent of plasticity would be greater in younger animals and reduced in older animals. Our baseline studies had focused on adult Long Evans male rats, 3 months of age. To extend this work, we studied young rats, 1 month of age, and older rats, 12 and 18 months of age. Animals of each age were exposed to short-day, long-day, and balanced-day light–dark cycles for 1 week, in the same photochambers with the same intensity of illumination. Brains were perfused, collected, sectioned, and immunostained for neurotransmitter and epigenetic markers. Because 18-month-old rats display more than 50–70% mortality before they reach (for rats) this old age, generating a sufficient number of aged rats for our experiments was a limiting step. We solved the problem working closely with the staff of our approved vendor, Harlan Laboratories, who were

able to generate aged rats by setting aside 40 rats in order to yield at least 10 aged animals. Normally distributed data were analyzed using two-tailed Student's t-test and one-way, two-way or repeated measures ANOVA, followed by post hoc comparison. All data were analyzed with IBM SPSS 24.0 (Chicago, IL) and represented by bar plot with mean and SEM (standard error). Significance in the figures was indicated with $*p \leq .05$; $**p \leq .01$; $***p \leq .001$. Exact $p$ and $t$-test values are reported for each experiment.

## 3 | RESULTS

### 3.1 | Photoperiod-induced dopamine plasticity declines with age

We hypothesized that the extent of neurotransmitter plasticity would be greater in younger animals and reduced in older animals. Our previous studies focused on adult male rats, 3 months of age (Dulcis et al.,

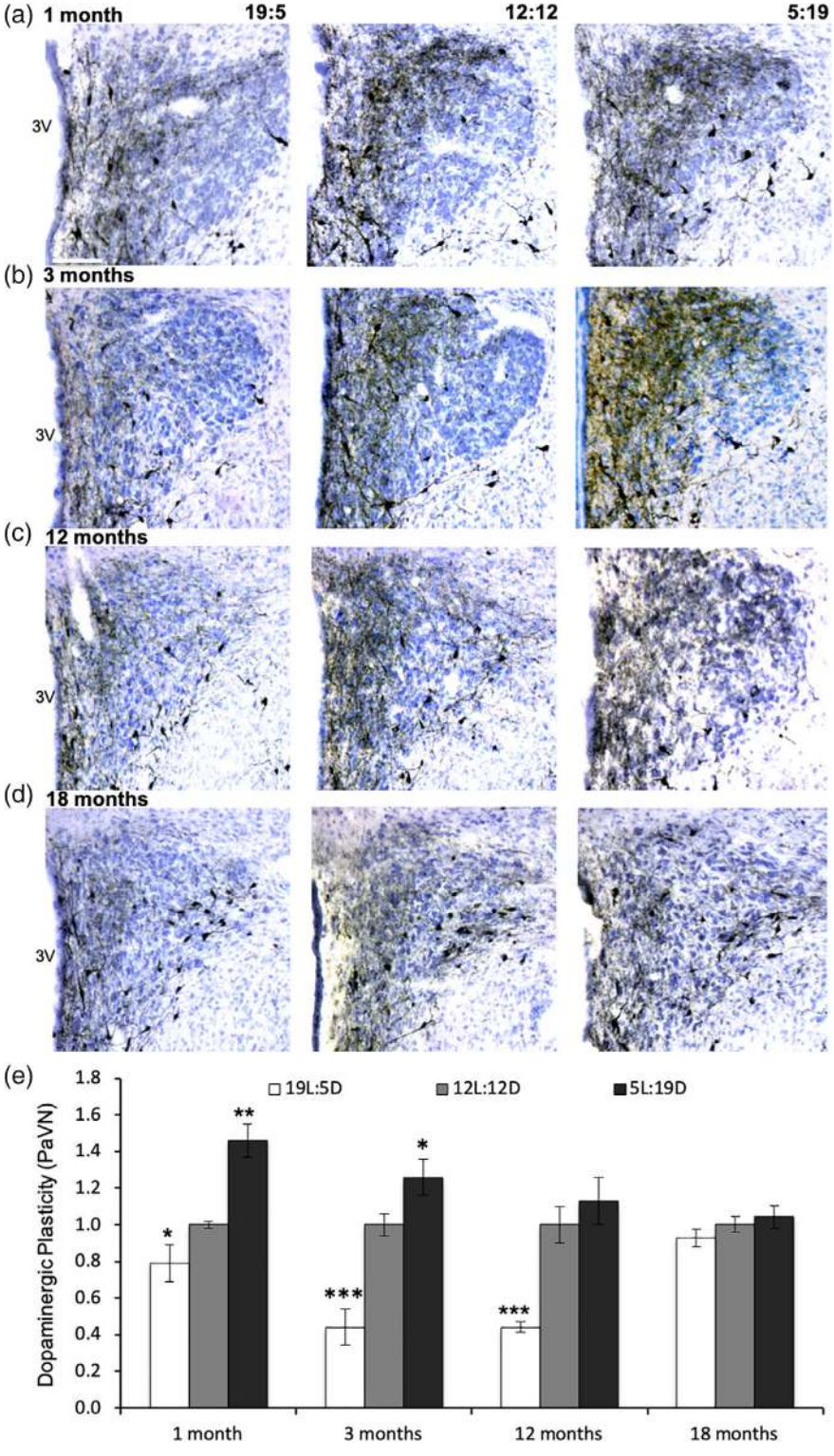


**FIGURE 1** Photoperiod-induced dopamine plasticity declines with age. (a–d) Bright-field images of PaVN sections immunostained with tyrosine hydroxylase (TH) antibody labeled with DAB (black) and counterstained with Giemsa (blue). Owing to the lateral extent of the PaVN only one side of the brain is shown. Exposure to short-day (5L:19D, right) and long-day (19 L:5D, left) photoperiods for 1 week alters the number of TH+ neurons in the PaVN relative to control animals (12L:12D, middle) in an age-dependent manner. (e) The number of TH+ neurons in the PaVN was compared among age groups using a normalization index of dopaminergic plasticity. Younger animals at 1 ($N$ = 6) and 3 months ($N$ = 10) showed a higher responsiveness to photoperiod, exhibiting a marked increase in number of TH + neurons when exposed to a short-day light cycle and a significant decrease in number when exposed to a long-day cycle. At 12 months of age ($N$ = 8), rats displayed a significant decrease in TH+ neurons following long-day light cycle relative to controls, but no change when exposed to a short-day light cycle. At 18 months ($N$ = 6), the photoperiod effect was lost and there were no changes in the number of TH+ neurons across photoperiods. Scale bar, 100 μm. Values are mean ± SEM. $**p = \leq .01$). 3 V, third ventricle [Color figure can be viewed at wileyonlinelibrary.com]

2013). To extend this work, we included the analysis of younger rats, 1 month of age, as well as older rats, 12 and 18 months of age. Animals of each age group were exposed to short-day (5 L:19D), long-day (19 L:5D), and balanced-day (12 L:12D) light–dark cycles for 1 week. Our findings indicate that there is a decline of dopamine plasticity with aging. To compare changes across photoperiods and age groups, we calculated a plasticity index. The mean number of TH+ or SST+ neurons in the balanced-day control photoperiod for each age group was normalized to 100% and given a plasticity value of 1; all other mean values corresponding to short- or long-photoperiods were divided by the mean from the control photoperiod of their age group. Exposure to short- and long-day photoperiods affected the number of TH+ neurons in the PaVN compared to control animals. Juvenile animals (1 month old) were the most responsive age group, displaying the highest TH plasticity index in response to short-day photoperiod exposure (Figure 1a,e), compared to adults (Figure 1b,e). Long-day

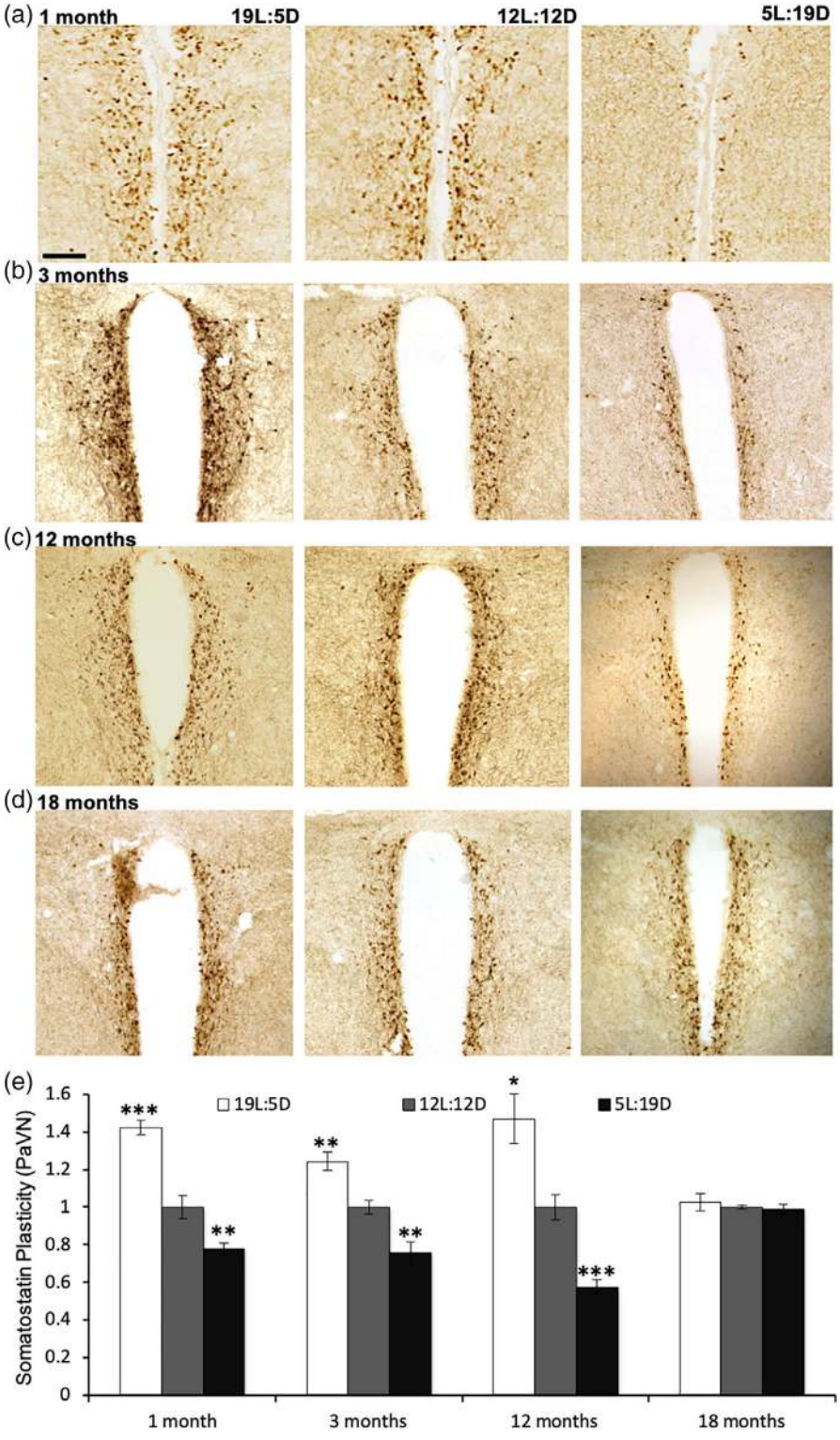


**FIGURE 2** Photoperiod-induced somatostatin plasticity declines with age. (a–d) Bright-field images of PaVN sections immunostained with somatostatin (SST) antibody labeled with DAB (brown). Exposure to short-day (5L:19D, right) and long-day (19L:5D, left) photoperiods for 1 week alters the number of SST+ neurons in the PaVN relative to control animals (12L:12D, middle) in an age-dependent manner. (e) The number of SST+ neurons in the PaVN was compared among age groups using a normalization index of somatostatin plasticity. Animals at 1 ($N$ = 6), 3 ($N$ = 8), and 12 ($N$ = 8) months of age showed photoperiod-dependent neurotransmitter switching in an inverse relation with respect to TH plasticity. As seen for TH expression, the effect was lost at 18 months of age ($N$ = 6) showing no change in the number of SST+ neurons across all photoperiods. Scale bar, 100 μm. Values are mean ± SEM. **$p$ = ≤.01; ***$p$ = ≤ .001 [Color figure can be viewed at wileyonlinelibrary.com]

exposure significantly reduced the number of TH+ cells in the PaVN of animals at 1 (Figure 1a,e) ($t_{(10)}$ = 2.45, $p$ = .0087 unpaired), 3 (Figure 1b, e) ($t_{(21)}$ = 5.11, $p$ = .000023, unpaired), and 12 months of age (Figure 1c,e) ($t_{(12)}$ = 4.48, $p$ = .0007, unpaired). Conversely, short-day exposure significantly increased the number of TH+ cells in the PaVN at 1 (Figure 1a,e) ($t_{(10)}$ = 3.25, $p$ = .0204, unpaired) and 3 months of age (Figure 1b,e) ($t_{(21)}$ = 2.55, $p$ = .0204, unpaired). However, this effect was lost at 12 and 18 months (Figure 1c–e). Interestingly, rats at 12 months of age showed a significant decrease in dopaminergic neurons only in response to long-day periods but no longer exhibited the increase with short-day exposure (Figure 1c,e). In aged rats (18 months), photoperiod induced TH-induced plasticity was completely abolished (Figure 1d,e). These findings demonstrate that young animals show greater photoperiod-induced neuroplasticity than old rats. Moreover, mature rats (3–12 months old) are more susceptible to light-induced dopamine switching in response to a stressful stimulus (long-day photoperiod) than they are to a more pleasurable environment (short-day photoperiod). We observed no overall anatomical changes in the PaVN between young and older adult rats.

### 3.2 | Photoperiod-dependent SST plasticity is also abolished with aging

Consistent with previous studies (Dulcis et al., 2013), short- and long-day exposure significantly influenced the number of SST+ neurons in the PaVN in an inverse relationship with the number of TH+ neurons. Long-day exposure significantly increased the number of SST+ cells in the PaVN in animals at 1 ($t_{(10)}$ = 5.31, $p$ = .0003, unpaired), 3 ($t_{(12)}$ = 3.55, $p$ = .0039, unpaired) and 12 ($t_{(12)}$ = 2.84, $p$ = .0146, unpaired) months of age (Figure 2a,b,c,e). Short-day exposure had the opposite effect in animals of these three age groups (1 month, $t_{(10)}$ = 2.99, $p$ = .010, unpaired; 3 months, $t_{(10)}$ = 3.52, $p$ = .0055, unpaired; 12 months, $t_{(12)}$ = 5.78, $p$ = .000043, unpaired), in which the number of SST+ cells in the PaVN was greatly reduced as the number of TH+ neurons was increased. Unlike photoperiod-dependent TH plasticity, short-day exposure in 12-month-old animals induced SST neuroplasticity (Figure 2c,e). The persistence of SST plasticity in these animals after TH plasticity has been lost, suggests that the loss of TH plasticity is not due to apoptosis. Consistently, the absolute number of TH+ neurons in animals of 12 months (3,419 ± 564, mean ± SE) and 18 months (3,494 ± 190, mean ± SE) of age is not lower than in 3-month old rats (3,240 ± 193, mean ± SE) exposed to control photoperiod. As observed for TH+ neurons, photoperiod-induced SST switching was completely abolished in animals at 18 months of age (Figure 2d,e). These data indicate that photoperiod-induced transmitter plasticity is age dependent.

### 3.3 | Patterns of Dnmt3a and acetyl H3 mRNA levels change with photoperiod exposure

To investigate the epigenetic mechanisms that may contribute to photoperiod-induced neurotransmitter respecification, we performed qRT-PCR of hypothalamic paraventricular tissue of 3 month old rats to identify changes in transcriptional regulation of methyl- and acetyl-

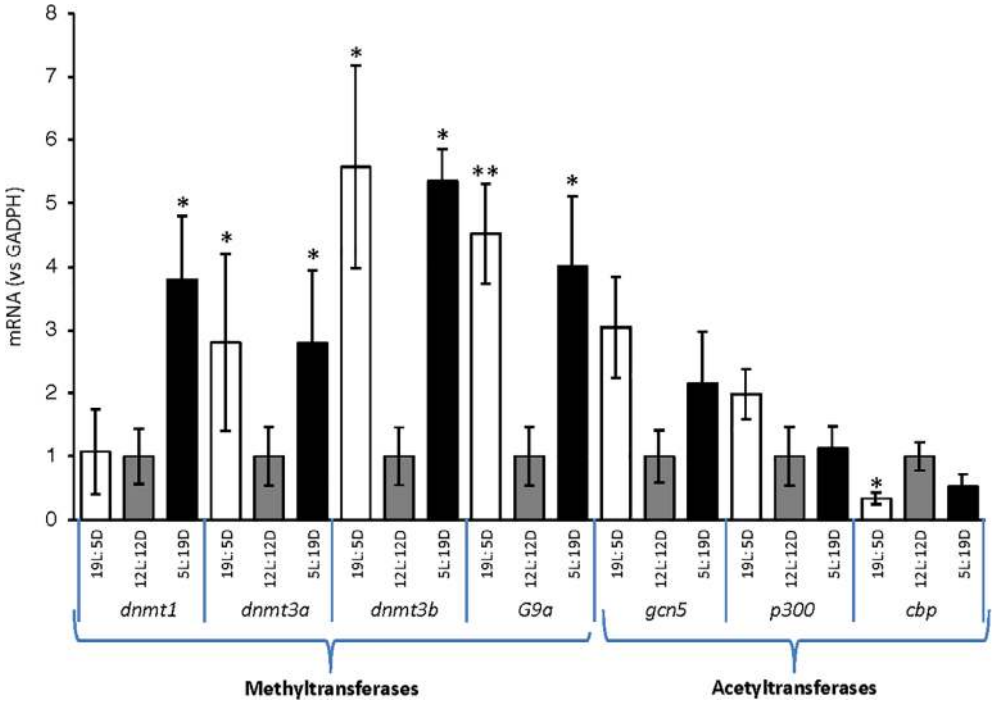


**FIGURE 3** Transcriptional regulation of methylases and transferases in response to altered photoperiod. Photoperiod regulates the mRNA levels of specific DNA methyl- and histone acetyltransferases in the PaVN. Quantification shows significant changes of DNA transferase transcripts (vs GADPH) compared to balanced (12L:12D) photoperiod ($N$ = 5 3-month-old rats per group). Values are mean ± SEM. *Dnmt1* (5L:19D, $t_{(8)}$ unpaired = 2.55, $p$ = .034); *Dnmt3a* (19L:5D, $t_{(8)}$ unpaired = 2.61, $p$ = .031); *Dnmt3b* (5L:19D, $t_{(8)}$ unpaired = 2.97, $p$ = .018; 19L:5D, $t_{(8)}$ unpaired = 2.75, $p$ = .025); *G9a* (5L:19D, $t_{(8)}$ unpaired = 2.48, $p$ = .038; 19L:5D, $t_{(8)}$ unpaired = 3.84, $p$ = .005); *CBP* (19L:5D, $t_{(8)}$ unpaired = 2.77, $p$ = .024) [Color figure can be viewed at wileyonlinelibrary.com]

transferases, *Dnmt1*, *Dnmt3a*, *Dnmt3b*, *G9a*, *GCN5*, *P300*, *CBP*, which have been shown to be regulated in association with various forms of neuroplasticity (Laplant et al., 2010; Maze, Covington, Dietz, et al., 2010; Park, Lim, & Shukla, 2012; Wu et al., 2017). We measured strong mRNA upregulation of all methyltransferases in response to altered photoperiods (Figure 3). We selected DNMT3a methyltransferase for further study because it introduces de novo methylation into otherwise unmodified DNA (Moore et al., 2013). In contrast, DNMT1 methylates hemimethylated DNA to maintain existing methylation patterns (Booth & Brunet, 2016; Jones & Liang, 2009). Acetyltransferases *gcn5* and *p300* did not display regulation in response to altered photoperiods; however, *cbp* showed a significant regulation selectively in response to the long-day photoperiod (Figure 3).

### 3.4 | The number of neurons expressing DNMT3a becomes photoperiod-independent with aging

We then investigated protein expression of a candidate methyl-transferase, DNMT3a, in TH+ and SST+ neurons that display neurotransmitter plasticity in the PaVN and compared the results across age groups and photoperiods (Figure 4a). Animals at 1 (TH, $t_{(4)} = 2.87$, $p = .0452$, unpaired; SST, $t_{(4)} = 1.00$, $p = .3739$, unpaired) and 3 months (TH, $t_{(4)} = 2.93$,

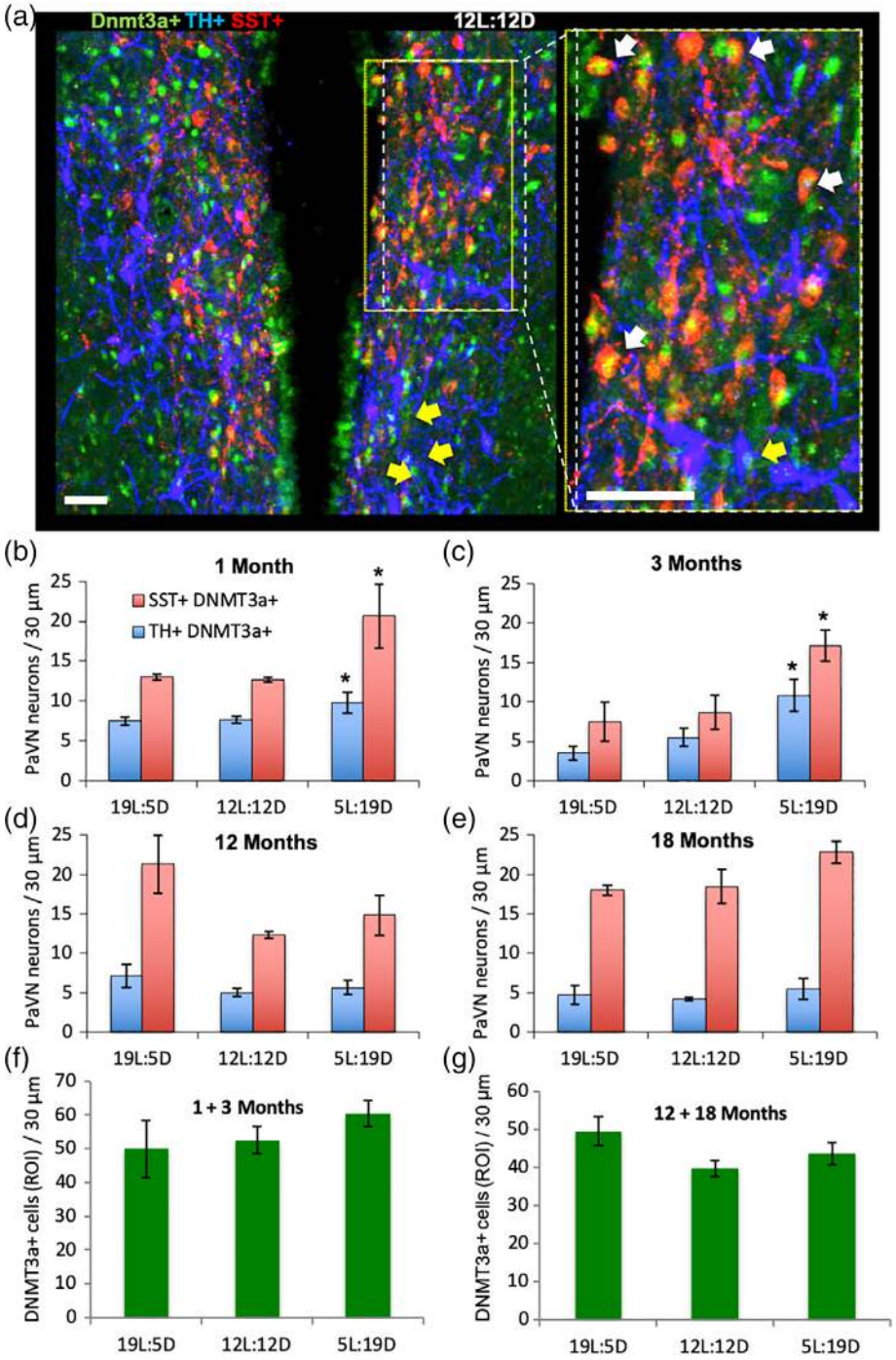


**FIGURE 4** DNMT3a expression is age-dependent and increases with exposure to short-day photoperiod. (a) Immunofluorescence micrograph showing a representative image of the PaVN (control photoperiod) immunostained to label TH+ (blue), SST+ (red), and Dnmt3a + (green) neurons. Scale bar, 100 µm. The inset (right) shows a higher magnification of the same region of the PaVN (left, dashed line) and SST+ Dnmt3a + (white arrows) and TH+ Dnmt3a + (yellow arrows) neurons. (b,c) An increase in DNMT3a expression in both TH+ and SST+ neurons following short-day photoperiod exposure was observed in animals at 1 and 3 months (*$p = \leq .01$) of age ($n = 3$/group). (d,e) The correlation of Dnmt3a level with photoperiod was lost in 12 and 18 month old rats. (f,g) There were no overall changes in the total number of DNMT3a + neurons (ROI) across photoperiods when compared within age-matched groups ($N = 3$ animals/group) [Color figure can be viewed at wileyonlinelibrary.com]

$p$ = .0424, unpaired; SST, $t_{(4)}$ = 2.90, $p$ = .0439, unpaired) had a significant increase in the number of both TH+ and SST+ cells that colocalized with DNTM3a in the PaVN following short-day exposure only (Figure 4b,c). Long-day exposure showed no difference in DNMT3a + TH+ or SST+ neurons from balanced-day controls. The photoperiod effect on DNMT3a coexpression in TH+ and SST+ cells in the PaVN was lost in rats at 12 and 18 months of age and was replaced by a marked overall increase (12 months, SST, $t_{(10)}$ = 2.23, $p$ = .04, unpaired; 18 months SST, $t_{(10)}$ = 4.95, $p$ = .0006, unpaired) in the number of DNMT3a + SST+ cells that was photoperiod-independent (Figure 4d,e) when compared to 3-month-old controls (12L:12D). The aging effect on the number of Dnmt3a + TH+ neurons in 12 and 18 month animals was characterized by a low basal level that was not significantly different (12 months, TH, $t_{(9)}$ = 1.45, $p$ = .18, unpaired; 18 months TH, $t_{(10)}$ = 0.4284, $p$ = .67, unpaired) from the one displayed by 3-month-old controls (12L:12D). To investigate whether different photoperiods were correlated with an altered number of DNMT3a + cells in the PaVN, we quantified the total number of DNMT3a + cells per 200 × 200 μM Region of Interest (ROI). Since animals at 1 and 3 months were the only age groups to exhibit significant changes, DNMT3a quantification was divided into two groups; younger

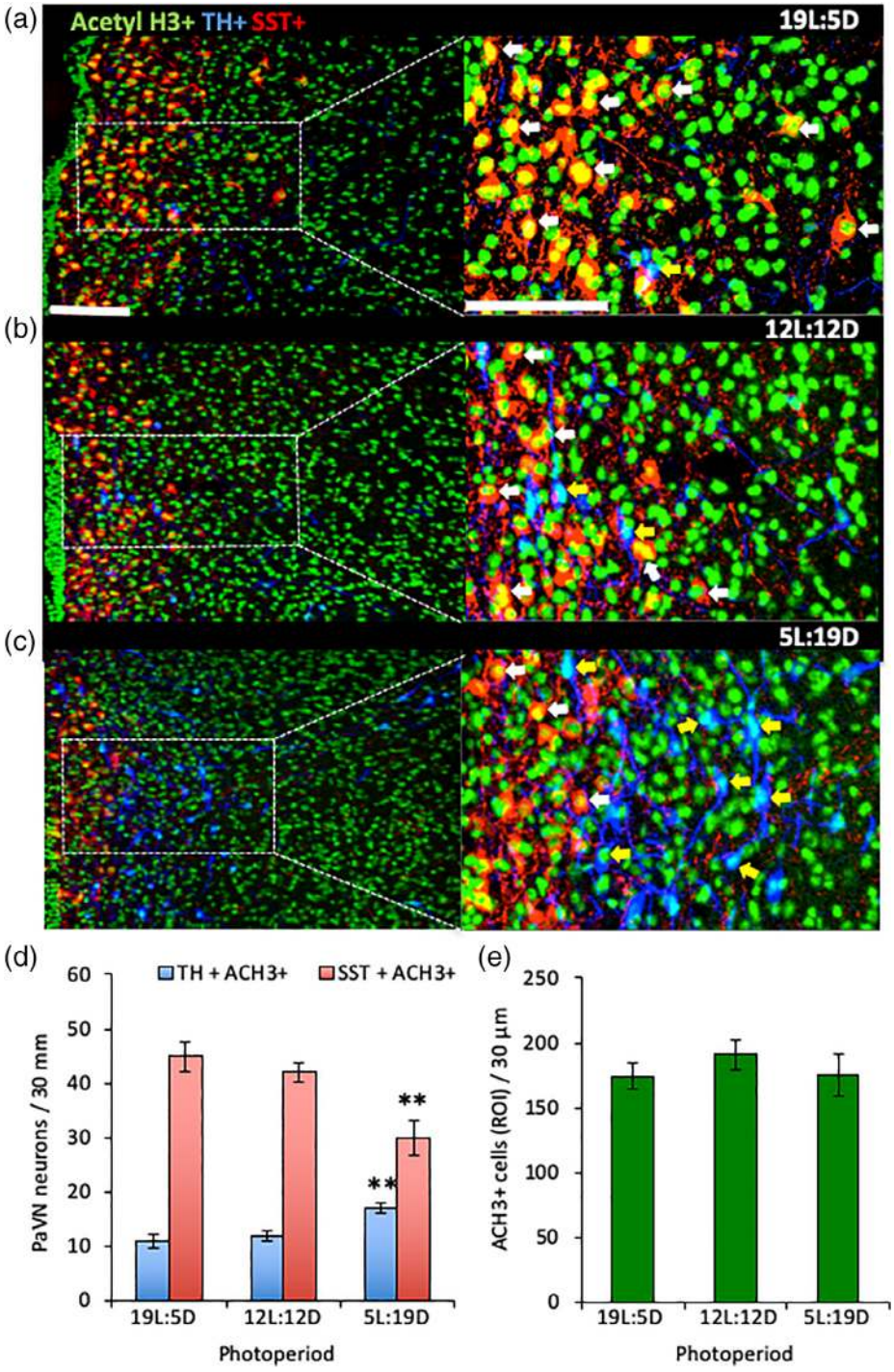


**FIGURE 5** Short-day photoperiod induces reciprocal levels of histone-3 acetylation in SST+ and TH+ neurons. (a–c) Confocal micrographs showing representative images of the PaVN of 3-month old rats exposed to long-, balanced-, and short-day photoperiods. Tissue was immunostained to label TH+ (blue), SST+ (red), and acetyl H3+ (green) neurons. Scale bar, 100 μM. The insets (right) show a higher magnification of the same regions of the PaVN (left, dashed line) with SST+ acetyl H3+ (white arrows) and TH+ acetyl H3+ (yellow arrows) neurons. (d) While the number of H3-acetylated SST+ and TH+ neurons increased following short-day photoperiod exposure, there was no significant change after exposure to the long-day photoperiod when compared to controls ($n$ = 5 animals/group). (e) There was no overall change in the total number of histone-3 acetylated neurons (ROI) across photoperiods ($N$ = 5 animals/group). Scale bars, 100 μm. **$p$ = ≤.001 [Color figure can be viewed at wileyonlinelibrary.com]

(1 and 3 months) and older (12 and 18 months). Our quantification showed no changes in the total number of DNMT3a + cells in the PaVN across photoperiods (Figure 4f, g), a result that may be achieved by simultaneous upregulation and downregulation of DNMT3a expression in different subpopulations of PaVN neurons. Furthermore, while *dnmt3a* mRNA levels increased both in short and long photoperiods (Figure 3), the number of DNMT3a immunopositive cells did not change, indicating that the observed photoperiod-dependent transcriptional regulation of *dnmt3a* is not paralleled by translational regulation of DNMT3a. Other potential mechanisms could be that the increase in dntm3a mRNA level is accompanied by an increase in protein DNMT3a levels but within the same number of cells or that some cells express more than in the balanced photoperiod and some express less resulting in comparable number of immunopositive cells across photoperiods. To compare changes in levels of mRNA and protein, Western blot assays for the protein would need to be implemented.

### 3.5 | Reciprocal H3 acetylation in SST+/TH+ cells following short-day photoperiod

To investigate further the epigenetic mechanisms of DNA accessibility associated with photoperiod-induced neurotransmitter switching, we performed immunohistochemistry to label PaVN TH+ and SST+ neurons with anti-AcetylH3, a marker for histone acetylation at the N-terminus (Figure 5a–c). To this aim, we focused on 3-month old rats, because they display high photoperiod-dependent plasticity as well as changes in Dnmt3a expression in both SST+ and TH+ phenotypes. Our data showed reciprocal H3 acetylation in TH+ versus SST+ neurons of the PaVN following short-day exposure (Figure 5c,d) (TH, $t_{(8)}$= 3.41, $p$ = .0092, unpaired; SST, $t_{(8)}$= 3.57, $p$ = .0073, unpaired). Specifically, short-day exposure increased the number of TH+ cells and decreased the number of SST+ cells acetylated at histone 3, relative to balanced-day controls (Figure 5b,d). As observed with the DNMT3a data, no difference was found in the total number of SST+ and TH+ neurons exhibiting histone 3 acetylation after long-day photoperiod exposure, compared to balanced-day controls (Figure 5a,d). To investigate whether overall levels of H3 acetylation in the PaVN were correlated with different photoperiods, we quantified the total number of AcetylH3+ cells per 200 × 200 μM ROI. Quantification showed no changes in the total number of AcetylH3+ cells in the hypothalamic PaVN across photoperiods (Figure 5e), suggesting differential upregulation and downregulation in histone acetylation in selected cell clusters or networks. Such circuit-specific epigenetic regulation could be detected only when the analysis included cellular specificity of expression of TH or SST.

## 4 | DISCUSSION

This study extends our previous findings on photoperiod-induced neurotransmitter respecification in the adult brain (Dulcis et al., 2013) by determining how this novel form of neuroplasticity is maintained or extinguished with aging. It also investigates possible epigenetic mechanisms underlying this phenotypic switch in neurotransmitter expression. We found clear age-dependent effects on photoperiod-induced neurotransmitter respecification as well as distinct changes in levels of DNMT3a that catalyzes DNA methylation and changes in histone acetylation in neurons identified by their neurotransmitter expression.

Our results reveal that age plays an important role in the ability of the nervous system to adapt to changes in photoperiod by displaying neurotransmitter plasticity. We found that young rats showed the most robust response to altered photoperiods by up or down-regulating the number of TH+ neurons in the PaVN. However, at 12 months of age, dopaminergic plasticity is displayed only in response to long-day photoperiod exposure (a stressor for nocturnal animals); the ability to increase the numbers of TH+ neurons when exposed to short-day photoperiod has been lost. Since increased levels of dopamine have been shown to mediate anxiolytic and anti-depressive effects when animals were tested for stress responses (Dulcis et al., 2013), our findings indicate that aging animals are less responsive to positive stimuli while still susceptible to stressors. This result is further supported by the *finding* that older rats (12–15 months) are more vulnerable to stress and are more susceptible to developing anhedonia than younger rats (3–5 months) (Herrera-Pérez, Martínez-Mota, & Fernández-Guasti, 2008). The increased prevalence of anhedonia among older rats was associated with neuroendocrine changes that occur with aging. Interestingly, we found that TH and SST plasticity did not follow the same time course of decline; SST switching is retained in both photoperiod conditions through 12 months of age. This result indicates that the factors mediating age-dependent gene regulation in response to seasonal changes in photoperiod affect neurotransmitter expression differently. At 18 months, both TH and SST plasticity were abolished. The decline of transmitter plasticity in the aging brain may be linked to the documented age-related decrease in the incidence rates of SAD (Kurlansik & Ibay, 2012). The reciprocal shift in TH and SST expression identifies a mechanism by which neural networks can adapt their functions in response to stressful or pleasant environments (Dulcis et al., 2013; Vogels, Sprekeler, Zenke, Clopath, & Gerstner, 2011) and may lead to nonpharmaceutical treatments for age-related pathologies associated with dopamine dysfunction.

Other forms of synaptic plasticity such as LTP and LTD are also impacted during aging (Kumar & Foster, 2018; Norris, Korol, & Foster, 1996). Much research has focused on the hippocampus, a brain region critically involved in learning and memory, which is particularly susceptible to dysfunction during senescence. Brain aging is associated with altered glutamatergic neurotransmission and $Ca^{2+}$ dysregulation (Kumar & Foster, 2018) that are linked to cognitive impairment. Aged animals show enhanced induction of LTD at hippocampal CA3-CA1 synapses, as a result of differences in calcium homeostasis between young and old rats (Norris et al., 1996). Deficits in synaptic plasticity during normal aging are also attributable to defects in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) trafficking (Norris et al., 1996). In addition, alterations in the size and stability of spines and boutons have been observed in the cortex during brain aging (Mostany et al., 2013). These structural changes result in

weaker synapses that are less capable of short-term plasticity in aged individuals reducing circuit function adaptability to a changing environment. The broad umbrella of mechanisms through which aging can negatively influence cognition now includes the impact of age-dependent decline and loss of neurotransmitter switching in addition to reduction in synapse strength and number.

The CNS is constantly being shaped by neural activity throughout the lifetime and epigenetic modifications are important regulators of neural plasticity (Tognini, Napoli, & Pizzorusso, 2015). To begin to examine whether epigenetic modification in TH+ and SST+ neurons involved in photoperiod-induced transmitter switching changed with aging, we investigated changes in mRNA levels and protein expression of DNMT3a that enables DNA methylation and evaluated histone acetylation in hypothalamic PaVN neurons.

We found that younger rats, at 1 and 3 months of age, had an increase in DNMT3a expression in TH+ and SST+ neurons in the PaVN following the short-day photoperiod exposure only. Intriguingly, de novo DNMT3a expression in this photoperiod revealed an increase in both TH+ and SST+ neurons instead of differential expression patterns predicted from their reciprocal induction of transmitter switching. DNMT3a activity at nonpromoter regions causes increased expression of neurogenic genes by hindering cytosine-phosphate-guanine (CpG) binding and gene repression (Lardenoije et al., 2015). Methyltransferases have been associated with neural plasticity (Laplant et al., 2010; Maze et al., 2010). Given that methylation has site-specific opposing effects on gene expression, future work will determine the mechanism through which DNMT3a acts in TH- and SST-expressing neurons. As age increased, the pattern of photoperiod-dependent DNMT3a localization in both phenotypes was lost and instead marked by an overall increase in de novo DNMT3a expression in SST+ cells across all photoperiods. Methylation patterns are known to change with aging and can result in local promoter-specific hypermethylation (Johnson et al., 2012; Jung & Pfeifer, 2015). The overall increase in DNMT3a expression in SST+ cells of the PaVN may be attributable to polycomb loss, because unmethylated regions bound by polycomb complexes have been shown to become methylated with aging (Cedar & Bergman, 2012; Jung & Pfeifer, 2015). This process would increase access to DNA and allow methylation levels to increase over time, resulting in a loss of plasticity. However, in younger rats, DNMT3a may be inhibiting CpG binding (Lardenoije et al., 2015) to increase plasticity and increase neurogenic gene activity. Total DNMT3a expression was quantified in the PaVN and compared across photoperiods within age groups and revealed no differences in overall levels, pointing to changes at the circuit level rather than overall expression. The photoperiod-dependent change in DNMT3a mRNA levels of 3 –month-old rats (Figure 3) in the absence of change in the number of DNMT3a positive cells (Figure 4) may represent an example of posttranscriptional regulation, as observed in olfactory-induced neurotransmitter plasticity mediated by miRNA inhibition of target transcripts (Dulcis et al., 2017). However, it is possible that higher levels of DNMT3a are present in cells immunopositive for DNMT3a and lower levels in the immunonegative ones.

As with changes in DNMT3a expression in younger rats, significant changes in histone 3 (H3) acetylation were observed only following short-day photoperiod exposure. However, TH+ and SST+ neurons exhibited a reciprocal pattern of H3 acetylation, different from that of de novo expression of DNMT3a. Short-day photoperiod exposure increased H3 acetylation in TH+ neurons and decreased H3 acetylation in SST+ cells. Because acetylation is typically associated with gene transcription, the findings were consistent with our hypothesis that acetylation levels of H3 would match the patterns of dopamine and SST neurotransmitter switching following short-day exposure. Histones H3 and H4 are particularly important in gene expression, because posttranslational modifications happening at these two histones are associated with transcriptional states (Annunziato, 2008; Yan & Boyd, 2006). For instance, recruitment of Nurr1, a transcription factor crucial for midbrain dopaminergic neuron development, to the promoter of TH gene has been shown to be enhanced by depolarization along with an increase of histone 3 acetylation in the Nurr1 binding regions of TH promoter (He et al., 2011). Total H3 acetylation quantified in the hypothalamus and compared across photoperiods revealed no differences in overall levels. However, identifying a reciprocal histone acetylation pattern with the increase in de novo DNMT3a expression in TH+ and SST+ neurons, in spite of a constant level of total H3 acetylation and DNMT3a expression, underscores the importance of studying epigenetic mechanisms at the circuit level and quantifying identified cell types. Changes in SST in the PaVN are matched by corresponding changes in mRNA levels and involve translation of de novo SST mRNA (Arancibia et al., 2000; Dulcis et al., 2013). While neurons are losing SST and being recruited to newly express TH, both translational silencing markers and pro-transcriptional markers may work in concert to stabilize the epigenome of these cells according to photoperiod. In summary, this study provides a map of TH/SST neuroplasticity across aging, shows an age-dependent pattern of DNMT3a expression across photoperiods and demonstrates a reciprocal H3 acetylation in neurons that switch their transmitter in the adult brain. Further work will more fully elucidate the roles of DNMT3a and H3 acetylation in sensory-stimulus induced neurotransmitter respecification via protein–DNA interactions.

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