HHS Public Access

Author manuscript

Biol Psychiatry. Author manuscript; available in PMC 2021 October 01.

Published in final edited form as:

Biol Psychiatry. 2020 October 01; 88(7): 566–575. doi:10.1016/j.biopsych.2020.04.013.

Stress regulation of sustained attention and the cholinergic attention system

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Abstract

Background: Stress exacerbates symptoms of schizophrenia and attention deficit hyperactivity disorder, which are characterized by impairments in sustained attention. Yet how stress regulates attention remains largely unexplored. Here we investigated whether a 6-day variable stressor (VS) altered sustained attention and the cholinergic attention system in male and female rats.

Methods: Sustained attention was tested with the sustained attention task (SAT). Successful performance on SAT relies on the release of acetylcholine (ACh) into the cortex from cholinergic neurons in the nucleus basalis of Meynert (NBM). Thus, we evaluated whether VS altered the morphology of these neurons with a novel approach using a Cre-dependent virus in genetically modified ChAT::Cre rats, a species used for this manipulation only. Next, electrochemical recordings measured cortical ACh following VS. Finally, we used RNAseq to identify VS-induced transcriptional changes in the NBM.

Results: VS impaired attentional performance in SAT and increased the dendritic complexity of NBM cholinergic neurons in both sexes. NBM cholinergic neurons are mainly under inhibitory control, so this morphological change could increase inhibition on these neurons, reducing downstream ACh release to impair attention. Indeed, VS decreased ACh release in the prefrontal cortex of males. Quantification of global transcriptional changes revealed that, although VS induced many sex-specific changes in gene expression, it increased several signaling molecules in both sexes.

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Disclosures

None of the authors have biomedical financial interests or potential conflicts of interest.

Conclusions: These studies suggest that VS impairs attention by inducing molecular and morphological changes in the NBM. Identifying mechanisms by which stress regulates attention may guide the development of novel treatments for psychiatric disorders with attention deficits.

Keywords

sustained attention; basal forebrain; morphology; gene transcription; prefrontal cortex; sex as a biological variable

Introduction

Attentional impairments characterize many psychiatric disorders, including attention deficit hyperactivity disorder (ADHD) and schizophrenia(1, 2). Psychiatric disorders are also stress sensitive, with stress causing onset and exacerbating symptoms, including attentional deficits(3–5). Yet how stress modulates attention is poorly understood. Several studies have investigated effects of the stress neuropeptide, corticotropin releasing factor (CRF), on rodent attention tasks. Central administration of CRF impairs both selective and sustained attention in rats(6, 7). Additionally, maternal separation stress decreases the number of omitted trials in an attention task, but not other performance measures(8). Surprisingly, no studies have investigated whether repeated stressor exposure during adulthood impacts attention.

One way to study sustained attention in rodents is with the sustained attention task (SAT), which tests rats' ability to monitor a situation for intermittent and unpredictable events(9). To succeed in the task, rats must distinguish between, and differentially respond to, signaled and non-signaled trials. An advantage of using SAT is that its underlying circuitry is fairly well-delineated and relies on the nucleus basalis of Meynert (NBM) in the basal forebrain(10). Lesions and optogenetic suppression of NBM cholinergic neurons disrupt performance on signaled trials, while optogenetic stimulation of these neurons enhances performance on signaled trials(11, 12). The phasic release of acetylcholine (ACh) into the medial prefrontal cortex (mPFC) is critical for signal detection(12, 13).

The present study aimed to determine how VS affected SAT and aspects of the underlying attention system in male and female rats. In other brain regions, stress can induce structural plasticity, so we assessed whether VS altered NBM cholinergic dendritic morphology. The effect of VS on ACh release in the mPFC was also evaluated. Finally, we quantified global transcriptional changes in the NBM following VS to identify molecular processes that could drive stress-induced changes in plasticity in the attention system.

Methods

Subjects

Procedures were approved by Temple University IACUC and consistent with NIH guidelines. Behavior and physiology studies used Sprague-Dawley rats (Charles River), given our history of studying stress and attention in this strain(7). Our technique to label NBM cholinergic neurons for morphology necessitated transgenic ChAT::Cre rats on a Long Evans background (Rat Resource and Research Center) that were genetically modified to

express a restricted recombinase-driver (Cre) in the presence of the choline acetyltransferase (ChAT) promoter(14). To link morphology changes to transcriptional changes, wild type Long Evans (Charles River) rats were used. Importantly, both the Sprague-Dawley and Long Evans strains engage their cholinergic system for sustained attention and both strains respond to repeated stressors similarly(15–17). All animals were adults (70+ days), maintained in a 12-hour light/dark cycle (lights off at 8:30am), and pair-housed with access to food and water *ad libitum*, except where otherwise noted.

Variable stress (VS)

Day 1 of VS, rats were subjected to 1h of restraint in their home cage in a Broome restrainer. Day 2, rats were exposed for 15min to 100μl of 2,3,5-Trimethyl-3-thiazoline (TMT), a synthetic fox odor, which was pipetted onto a non-woven sponge and taped to the inside wall of an empty mouse cage. Day 3, rats were subjected to 15min of forced swim in a cylinder (40cm high, 18.5cm diameter) containing water 30cm deep, maintained at 23–25°C. Rats were dried off and placed under a heat lamp for 30min following swim stress. Days 4, 5, and 6 these three stressors repeated in the same order. Rats assigned to the control condition were left undisturbed in their home cages Days 1–6 (Fig.1A).

SAT

Individually-housed adult male and female rats were food restricted to 85% of their free-feeding weight. Rats were trained in touchscreen SAT operant boxes as described in Supplementary Information (SI) and previously(18, 19). In brief, rats were trained to discriminate between signaled (durations 500ms, 50ms, or 25ms varied pseudorandomly) and non-signaled trials. Once they met criteria, VS groups (male n=13; female n=10) were then exposed to daily stressors and tested in SAT 30min after the cessation of each stressor. For the unstressed group (male n=16; female n=11), rats continued to run in SAT without any stressor exposure for 6 days after reaching criteria.

Attentional performance was assessed with the vigilance index, based on the proportion of hits and false alarms, such that a value of 0 indicated that the rat could not distinguish between signaled and non-signaled trials and a value of 1 indicated perfect performance (details in SI)(9).

Dendritic morphology

Rats underwent aseptic surgery as detailed in SI methods. The virus AAV9.CAG.Flex.eGFP.WPRE.bGH (from Dr. Hongkui Zeng of the Allen Institute for Brain Science via UPenn Viral Vector Core) was bilaterally infused at 1μL, 1×10^{9}gc/μL per side in the NBM as detailed (SI methods). Validation was done as described in SI with an anti-ChAT antibody and 92% of cells were positive for ChAT and the virus (SI Fig.1). Imaging analysis details are in SI methods. Group numbers are: unstressed male n=5 rats, n=26 cells; unstressed female n=4 rats, n=27 cells; male VS n=4 rats, n=22 cells; female VS n=4 rats, n=25 cells.

In vivo amperometric recordings

Electrodes were prepared as described in SI methods. All recordings were performed under anesthesia. Following the last manipulation, control (n=7 male, n=6 female) and VS (n= 6 male, n=6 female) rats were anesthetized with urethane (1.2–1.5g/kg,i.p), placed in the stereotax, and choline oxidase-coated microelectrodes were lowered into the right mPFC (AP: +3.0mm, ML: −0.7mm, DV: −2.7–3.0mm). A reference electrode was implanted in the rostral cortical region of the contralateral hemisphere. If microelectrodes failed to meet testing criterion during *in vitro* calibration, recording sessions were delayed. In these instances, rats were given additional stressor sessions for another 24–48h until a well-calibrated electrode was identified and recordings were completed. More details are in SI methods.

RNA extraction

A different cohort of rats was sacrificed by rapid decapitation 30min after the cessation of the final stressor in VS or the control procedure, mirroring the timing of the last behavioral test. The NBM was bilaterally dissected, frozen on dry ice, and stored at −80°C until RNA extraction (n=4/group) or qPCR validation (n=8/group). RNA was extracted as detailed in SI methods. RNA quality was assessed using Qubit RNA HS assay and BioanalyzerRNA6000 Nano assay. Libraries were prepared using NuGen Ovation RNA-Seq Systemv2 from total RNA and sequenced by UCLA Neuroscience Genomic Center (SI methods).

RNA-sequencing analysis and qPCR

Sequences were aligned to Rat Genome assembly (Rnor_6.0) using STAR-2.5.2a(20). rRNA reads were filtered using Bedtools intersectBed and rRNA annotation from Biomart. Gene read counts generated by HTseq-count were used to compute fold changes and significance of expression differences using DESeq(21). DEGs were assessed through a generalized linear model implemented in limma, with phenotype (VS vs. control) and sex (male vs. female) as main factors. The log expression values for each gene were averaged over treatment group, and the log2 fold change was computed. P-values were adjusted for multiple comparisons using Benjamin-Hochberg correction method. Heat maps of raw reads were generated using R's pheatmap function. Unsupervised clustering was performed by pheatmap in default settings.

qPCR was performed on a separate cohort of animals to validate the three DEGs that were most significant with the highest fold change following stress found in males and females (see SI Methods). Fold change was calculated using $2^{-\Delta\Delta Ct}$ analysis method.

Results

VS impairs SAT

Our main finding was that VS impaired attention in both sexes. A timeline depicting behavioral testing relative to the manipulations is shown in Figure 1A. Behavioral results were analyzed with mixed-factors ANOVAs. All analyses violated sphericity, so degrees of freedom were corrected with Greenhouse-Geisser estimates. Non-significant statistics reported in SI Results. *A priori* we predicted that differences in stress-induced attention

deficits would not emerge until the later days of stress exposure (e.g., Days 4–6). Therefore, planned comparisons (using LSD posthocs) between control and VS rats were conducted for each day of behavior testing.

VS exposure reduced the vigilance index (signal durations combined), such that there was a main effect of condition in male and female rats [$F(1,46)=6.95$, $p=.011$, $\eta^2_{partial} = .131$] (Fig. 1B). Similarly, VS impaired attention (main effect) at each stimulus duration: 500ms [$F(1,46)=8.56$, $p=.005$, $\eta^2_{partial} = .157$], 50ms [$F(1,46)=6.51$, $p=.014$, $\eta^2_{partial} = .124$], and 25ms[$F(1,46)=4.61$ $p=.037$, $\eta^2_{partial} = .091$] (Fig.1E–G). There were no effects of sex, sex×condition, nor sex×condition×day interactions for any of the vigilance index measures. These findings indicate that stress impairs attentional performance regardless of sex. Figure 1.B–D shows the vigilance index combined data, as well as separated by sex for transparency regarding sex as a biological variable. Based on *a priori* predictions, we also analyzed data to assess the effect of stress at each individual day of the manipulation and trends ($p \leq .10$ and $>.05$) and significant effects ($p<.05$) are indicated with pound signs and asterisks, respectively. Even though sex was not a significant factor in our analysis, these planned comparisons do suggest that TMT exposure on Day 5 disrupted performance in both sexes, while swim stress on Day 6 only affected males. These findings may indicate that either TMT is a more effective stressor for both sexes, or that females are quicker at recovering their attentional deficits after repeated stress.

Omissions were altered by VS and there was a day×condition interaction [$F(3.16, 145.42)=5.73$, $p=.001$, $\eta^2_{partial} = .111$] (SI Fig. 2). Full statistical analysis is reported in SI results. In short, omissions did not change over time in control rats, but changed in VS rats. Post-hoc tests revealed that omissions increased relative to baseline on days when rats were exposed to TMT and swim stress, but not restraint stress: days 2 ($p=.037$), 3 ($p<.001$), 6 ($p=.005$) with a trend for day 5 ($p=.056$). Increased omissions could indicate a disruption in attention or impaired motivation. Given that we did not see our overall measure of attentional performance, vigilance index, disrupted specifically by TMT and swim stress, it is more likely that these stressors decreased motivation to perform the task

VS alters NBM cholinergic dendritic morphology

VS induced dendritic hypertrophy in both sexes. Timeline for tissue collection (Fig. 2A). An image of a virally-labeled NBM cholinergic neuron in a ChAT::Cre rat (Fig. 2B). VS exposed ChAT::Cre rats had longer dendrites than control ChAT::Cre rats, as evidenced by a main effect of VS [$F(1, 96)=4.88$, $p=.030$, $\eta^2_{partial} = .048$], but no effect of sex nor interaction (Fig. 2C). A sex difference was found such that male dendrites had more nodes [$F(1, 96)=18.60$, $p<.001$, $\eta^2_{partial} = .162$] and ends [$F(1, 96)=24.68$,$p<.001$, $\eta^2_{partial} = .204$] than female dendrites (Fig. 2D,E). There were no main effects of VS or interactions for these measures.

Sholl analysis was conducted on intersections and dendritic length within the circles using a mixed factors ANOVA (sex×stress condition×distance) and revealed that VS induced dendritic hypertrophy. Representative Sholl analysis of neurons from each group (Fig. 2F–I).

As distance from the cell body increased, intersections [$F(12, 1152)=255.19$, $p<.001$, $\eta^2_{partial} = .727$] and length [$F(12,1152)=287.27$, $p< .001$, $\eta^2_{partial} = .750$] decreased. There were sex×Sholl interactions for intersections [$F(12, 1152)=12.15$, $p< .001$, $\eta^2_{partial} = .112$] and length [$F(12, 1152)=18.76$, $p< .001$, $\eta^2_{partial} = .163$]. LSD post-hoc tests revealed female dendrites had fewer intersections ($p<.05$, 40–60μm) and were shorter near the cell body ($p<.05$, 40–80μm), but had more intersections ($p<.05$, 180–260μm) and were larger further away from the cell body ($p<.05$, 200–260μm). This analysis revealed a main effect of VS on intersections [$F(1, 96)=4.18$, $p=.044$, $\eta^2_{partial} = .042$] and length [$F(1, 96)=4.78$, $p=.033$, $\eta^2_{partial} = .046$], such that stress increased both measures (Fig. 2J–M). No other main effects or interactions were significant.

Electrochemical recording results

Timeline for typical electrochemical recordings (Fig. 3A). Examples of amperometric traces in response to depolarization for males (Fig. 3B) and females (Fig. 3C). There were baseline sex differences and sex-specific stress effects on depolarization-evoked ACh release in the mPFC of Sprague-Dawley rats as illustrated by VS×sex interaction [$F(1,21)=6.37$, $p=.020$, $\eta^2_{partial} = .233$]. LSD post-hoc tests revealed a baseline difference in evoked release such that control males released more ACh than control females ($p=.023$). There was a sex-specific effect of VS, such that VS decreased evoked release in males ($p=.013$), but not females ($p=.385$) relative to their unstressed same-sex counterparts (Fig. 3D).

VS altered NBM gene transcription

Timeline for tissue collection (Fig. 4A). RNA-seq measured transcriptional changes in the NBM of control and VS rats. A heatmap of significantly regulated genes ($p<.05$) from the RNA-seq analysis (Fig. 4B,left). VS rats showed a distinct expression pattern compared to controls, (log2(FC)>1 or <−1; p<0.05). KEGG pathway analysis revealed VS regulated genes function in pathways such as Alanine, aspartate, and glutamate metabolism (Fig. 4B, right). KEGG pathway analyses were also conducted on genes altered by VS in males and females (i.e., sex specific) and many immune-related pathways were changed in both. Males had more pathways reach significance following VS than females (SI Table1). We also compared within sex, and heatmaps show significantly regulated genes for males and females (Fig. 4C–D). VS regulated more genes in female than in male rats (217 and 86, respectively) (Fig. 4E–F). Most genes were upregulated following VS in both females (73.7%) and males (63.95%). Across sexes, 14 genes were regulated in both male (16.28%) and female (6.45%) stressed animals (Fig. 4E–F). To validate our bioinformatic data, we selected the 3 most significantly VS-regulated genes in males (*Dusp1, Pdk4, and Klf4*) and the 3 most significantly VS-regulated genes in females (*Dusp1, Dusp10*, and *Rfc5*) and analyzed gene expression in a biological replicate cohort by qPCR in both sexes (Fig. 4G–K). qPCR confirmed differential expression of top genes, including the upregulation of *Dual Specificity Phosphatase 1* (*Dusp1)* in males and females, which was the highest upregulated gene in both sexes identified with RNAseq. qPCR also revealed that *Pdk4* and *Klf4* were upregulated by VS only in males, while *Dusp10* and *Rfc5* were upregulated by VS only in females, and thus represent sex-specific gene changes.

Discussion

Human studies have associated life stress with attentional impairments(22–24), but previously the repeated effects of stress on sustained attention were not systematically assessed. Here we found that a 6-day VS procedure impaired the SAT vigilance index in male and female rats and induced dendritic hypertrophy in the NBM cholinergic neurons that underlie this task. These VS-induced changes were accompanied by a decrease in mPFC ACh release only in males, perhaps driven by an increase in inhibitory inputs to NBM cholinergic neurons as a result of the observed dendritic hypertrophy. VS exposure also caused sex-specific gene expression changes in NBM neurons, with more genes being up- and down-regulated in females than in males. However, in both sexes, the top upregulated gene following VS exposure was *DUSP1*. DUSP1 alters dendritic morphology in other regions(25), so changes in its expression may contribute to VS-induced dendritic hypertrophy.

Stress and attention

Here we addressed, for the first time, whether repeated stress in adulthood impairs sustained attention. Compared to controls, VS impaired attention in SAT in both male and female rats, although attention deficits in females appear to recover by the last stressor exposure. During SAT, the signal duration varies and VS impaired performance on all signal durations, indicating that stress was disruptive even when the signal was relatively easy to detect (500ms). One feature of our design was testing rats on SAT 30min after each daily stressor exposure, which makes it difficult to dissociate acute vs. chronic stress effects. There was no drop in performance following the first stressor exposure, so one possibility is that repeated stressor exposures are required to elicit attentional deficits. Another consideration is that different stressors vary in their impact on attention. Future studies are needed to dissociate the effects of repeated stress vs. stressor type on attention in males and females. Even with these limitations, the consequences of the disruptive effect of stress on sustained attention are likely impactful. Sustained attention subserves other forms of attention, including selective and divided attention, and is critical for higher order cognitive processes(10, 26). Therefore, cognitive deficits caused by stress may result, in part, from difficulty sustaining attention.

The stress response is complex and involves central and endocrine changes. The two modulators most associated with stress are: 1) glucocorticoids, which are released through HPA axis activation, and 2) CRF, which is released to initiate the HPA axis, as well as centrally to coordinate behavioral responses to stress. Human studies have not found a relationship between glucocorticoid levels and attentional processes(27–29). In contrast, central administration of CRF impairs SAT in male and female rats, and other cognitive processes that rely on sustained attention(6, 7, 30, 31). It is therefore likely that CRF plays a role in mediating the stress effects observed here, but future studies are needed to test this idea.

Stress induces dendritic hypertrophy in NBM cholinergic neurons

Accurate performance on SAT requires the release of ACh into the mPFC from NBM cholinergic neurons(12, 13). Therefore, stress could impair SAT by affecting NBM cholinergic neurons. Repeated stress impacts other neuron types by altering dendritic morphology(32–38). Existing tools to assess dendritic morphology in the basal forebrain have limitations: Golgi impregnation does not allow for the cell-type specificity; antibody staining does not fully label processes(39); and biocytin filling requires whole cell recording and only labels a small number of neurons that could be successfully patched(40). Thus, only a total of three basal forebrain cholinergic neurons from male rats had previously been reconstructed for morphology(40). In contrast, our approach of virally labeling cholinergic neurons in ChAT::Cre rats allows for cholinergic specificity, clear visualization of processes, and labeling of a large population of NBM cholinergic neurons. One caveat is that a recent publication found increased copies of the vesicular acetylcholine transporter gene in the prefrontal cortex of ChAT::Cre rats(41). It is not clear if this change would affect cholinergic dendritic morphology, but ChAT::Cre rats were used for both the control and VS conditions.

We first discovered a baseline sex difference in the shape of NBM cholinergic dendrites, with male dendrites being more complex than female dendrites. However, VS had the same effect on NBM cholinergic dendrites in both sexes, increasing their length and complexity. This stress-induced dendritic hypertrophy likely resulted from the repeated nature of the stressor, because it is typically chronic stress that is required to alter structural plasticity(32–38). Most prior studies exploring the effects of chronic stress on dendrites have focused on the hippocampus, amygdala, and PFC and there are examples of repeated stressors causing atrophy and hypertrophy depending on the region, manipulation, time after stressor cessation, and sex of the animal(32–38). Fewer studies have assessed the effects of stress on dendrites in ascending arousal systems. However, we previously found that lifelong overexpression of CRF increased the complexity of noradrenergic dendrites in the locus coeruleus (LC)-arousal system in male mice(42). This effect was not observed in females. However, female LC dendrites are longer and more complex than those of males at baseline, so perhaps stress could not induce further hypertrophy(42, 43). Given the widespread effects of ascending arousal circuits on cognition and sleep/wake, more studies investigating whether stress can alter the morphology of other cell groups are warranted.

Sex-specific effects of stress on the release of ACh into the mPFC

Changes in dendritic morphology affect how inputs are processed. Most NBM inputs, including parvalbumin, neuropeptide Y, and somatostatin neurons, inhibit cholinergic neurons (44–47). Given the high proportion of inhibitory inputs onto NBM cholinergic neurons, dendritic hypertrophy induced by VS could increase the inhibitory tone on these cells, ultimately reducing ACh output. We found that VS reduced ACh release in males, as predicted. Surprisingly, we did not observe a stress-induced change in ACh release in females, even though VS increased complexity of their NBM dendrites. The cause of this discrepancy is unclear. It is possible that stress alters inputs into cholinergic neurons differently in males and females. Perhaps in females, VS increases presynaptic excitatory synapses or reduces presynaptic inhibitory synapses on NBM cholinergic dendrites, negating an impact of dendritic hypertrophy on downstream ACh release.

The fact that stressed and unstressed females had similar levels of ACh release in the mPFC, yet VS impaired attention, raises questions about the cause of the attentional impairment in females. Here we focused on NBM cholinergic neurons, given that they are critical for attention, yet little is known about they are impacted by how stress. However, the mPFC and GABAergic neurons in the NBM also modulate certain aspects of SAT performance(10, 48, 49). Perhaps there are stress-induced changes in these other cell types that drive the female deficit in attention following VS.

The focus of this project was on the mechanisms by which VS can impair attention. However, in the process of studying the effects of stress we also discovered important baseline sex differences in the cholinergic attention system. These discoveries result from the fact that most previous studies typically used male rodents in their design, although McGaughy and Sarter (1999) demonstrated that, like in males, NBM cholinergic neurons were necessary for accurate SAT performance in female rats(50). Interestingly, male rats exhibited higher evoked ACh release following terminal depolarization under control conditions as compared to the female rats. Given the critical role of ACh release in sustained attention, one might predict that males would be better at SAT than females. Yet several studies, including this one, have found no sex differences in baseline SAT performance(7, 18). Females may instead have a downstream compensatory mechanism that allows them to adequately sustain attention, despite lower ACh levels. Females have more cortical nicotinic and muscarinic ACh receptors than males(51–53). Thus, the female mPFC may be better at detecting ACh release than the male mPFC, keeping female attention similar to male levels, despite lower ACh release at baseline.

Sex differences in gene transcription

To identify molecular processes by which stress could alter NBM neurons, we used RNAseq to assess VS-induced transcriptional changes in the NBM. Given that VS caused dendritic hypertrophy in both sexes, we first collapsed across sex to assess gene transcription. Many genes involved in signaling were upregulated by stress. Consistent with this finding, pathway analysis revealed changes in several signaling pathways including: inositol phosphate metabolism, which regulates calcium signaling; PI3K-Akt signaling; and mitogen-activated protein kinase (MAPK) signaling.

To assess whether top candidate genes altered by VS were similar in males and females, we also analyzed the sexes separately. VS caused a greater number of gene expression changes in females than in males, and this was true for both up- and down-regulated genes. Although VS induced dendritic hypertrophy in both sexes, only 14 genes were regulated in a similar direction in males and females. These data could indicate that there are sex-specific molecular processes underlying stress-induced dendritic hypertrophy of cholinergic neurons. Such sex-specificity exists in the mechanisms by which estrogens increase neuronal excitability in the hippocampus(54, 55).

Alternatively, our analysis indicates that some genes are similarly altered by VS in both sexes, so these genes may be the ones critical for morphological changes. The top gene upregulated by stress for both sexes was *DUSP1*, also known as mitogen-activated protein kinase phosphatase-1. MAPKs, including p38, ERK and JNK, are signal transducing

enzymes that influence proliferation, differentiation, development, transformation, and apoptosis. DUSPs dephosphorylate MAPKs at threonine and tyrosine residues, inactivating their function(56, 57). DUSP1 is a stress-inducible protein that can regulate dendritic morphology(25, 58–60). Specifically, DUSP1 overexpression in developing dopaminergic neurons increases dendritic branching and length(25). Our results suggest that the VS-induced expression of *DUSP1* could promote cholinergic dendritic hypertrophy. Given that tissue collection occurred 30min after the final stressor cessation, it remains unclear whether transcriptional changes in the NBM result from acute vs. chronic stressor exposure. There is evidence that chronic stress increases DUSP1 in the ventrolateral orbital cortex and hippocampus(59, 60). Moreover, typically chronic stress causes changes in dendritic morphology and we believe that many of these transcriptional changes are linked to the observed changes in structural plasticity(32–38). It is therefore likely it is the chronic nature of the VS manipulation that increase *DUSP1* to promote dendritic hypertrophy. However, future studies are required to test this idea.

Conclusion

Stress impairs sustained attention in male and female rats. It also alters the morphology of cholinergic neurons in the attention circuit and induces sex-specific changes in cortical ACh release. Given that sustained attention underlies higher order cognitive processes and is disrupted in several psychiatric disorders, understanding the molecular processes by which stress impairs this attention system may lead to novel treatments to improve cognitive function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

An earlier version of the behavioral, molecular, and electrochemical data was published in an abstract in *Neuropsychopharmacology* for the 2018 American College of Neuropsychopharmacology meeting. We would like to thank Natalie Newcamp, Evelyn Ordoñes Sanchez, Kimberly Wiersielis, and Jessica Tucci for their technical assistance. This work was supported by T32 DA007237 (SRE), NIH K99/R00 MH092438 (DAB), NSF CAREER grant IOS-1552416 (DAB), NSF grant IOS- 1929829 (DAB), Pennsylvania Department of Health grant 420792 (DAB), iGEM Award for Genomics from Temple University (DAB), Charles E Kaufman Foundation Young Investigator Award (EAH), NIH-NIDA Avenir Director's Pioneer Award DP1 DA044250 (EAH), Brain and Behavior Research Foundation 570769 (EAH,) R00 MH090237 (MRA), and NIH AG046580 (VP).

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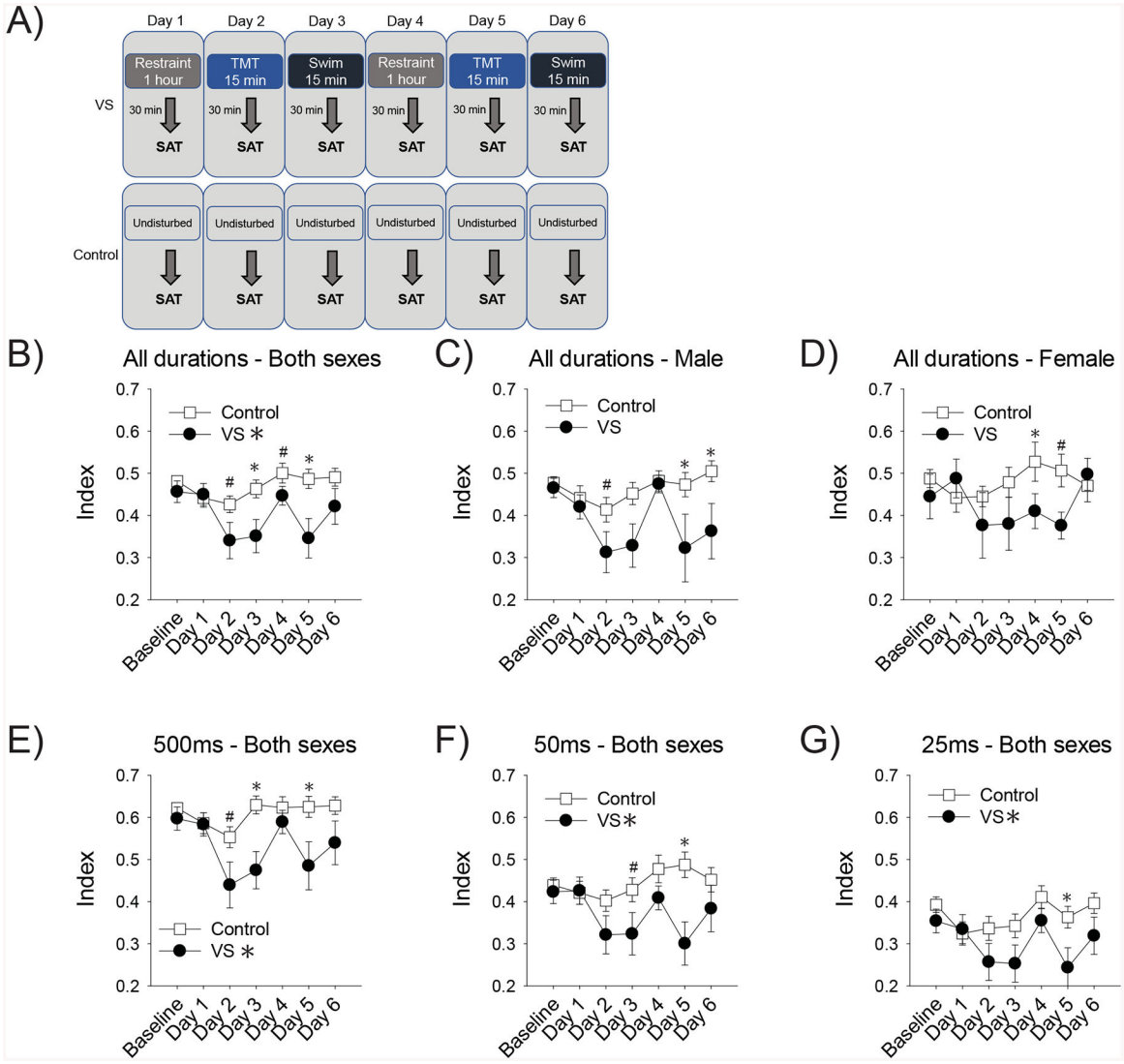

Figure 1. VS impaired SAT performance as assessed with the vigilance index.

Timelines depict the VS and control manipulation and when these occurred relative to behavior testing (A). For the graphs, the first baseline measure on the x-axis reflects an average of 3 days of performance prior to exposure to VS or the control manipulation in Sprague-Dawley rats. Then daily performance 30 min after stressor cessation is depicted. VS impaired the vigilance index, an overall measure of attentional performance, in both sexes (B). Although there were no effects of sex, we presented the data from males (C) and females (D) separately on this measure for transparency with regards to sex as a biological variable. The vigilance index data was also analyzed separately for each of the three signaled-trial stimulus durations. VS impaired vigilance index performance at 500ms (E), 50ms (F), and 25ms (G). Asterisks indicate $p < .05$ from the control group, pound signs indicate a trend ($p \leq .10$ and $> .05$)

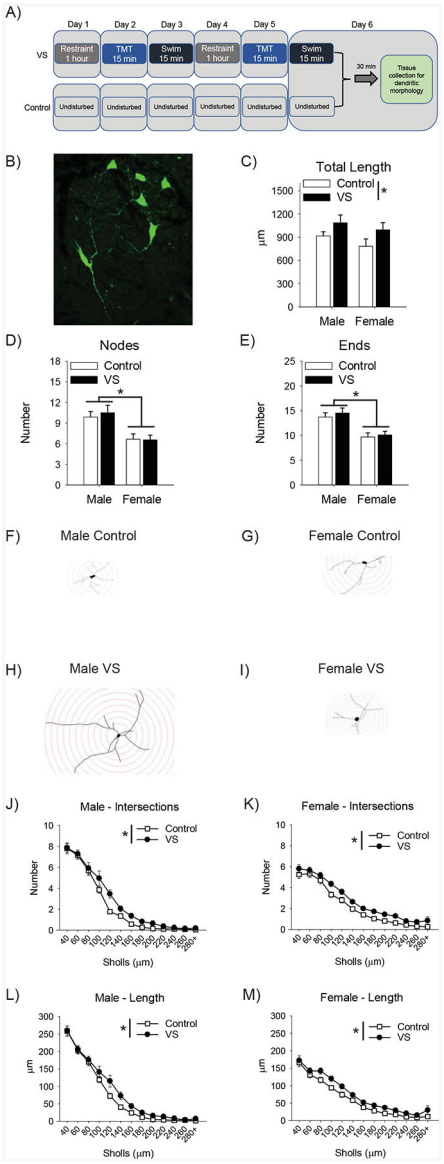

Figure 2. VS increased the complexity of dendrites from NBM cholinergic neurons.

Timelines depict the VS and control manipulation and the timing of tissue collection (A). Image of a NBM cholinergic neuron labeled with a Cre-dependent virus in a ChAT::Cre rat (B). VS increased the total length of dendrites in both sexes (C). There was also a main effect of sex, such that male NBM cholinergic dendrites had more nodes and ends than those of females (D,E). Sholl analysis was performed to assess complexity as a function of distance from the cell body and a representative trace from each group is depicted (F–I). VS increased the number of intersections with the circles in both male (J) and female (K) rats. VS also increased the length within circles in both sexes (L,M).

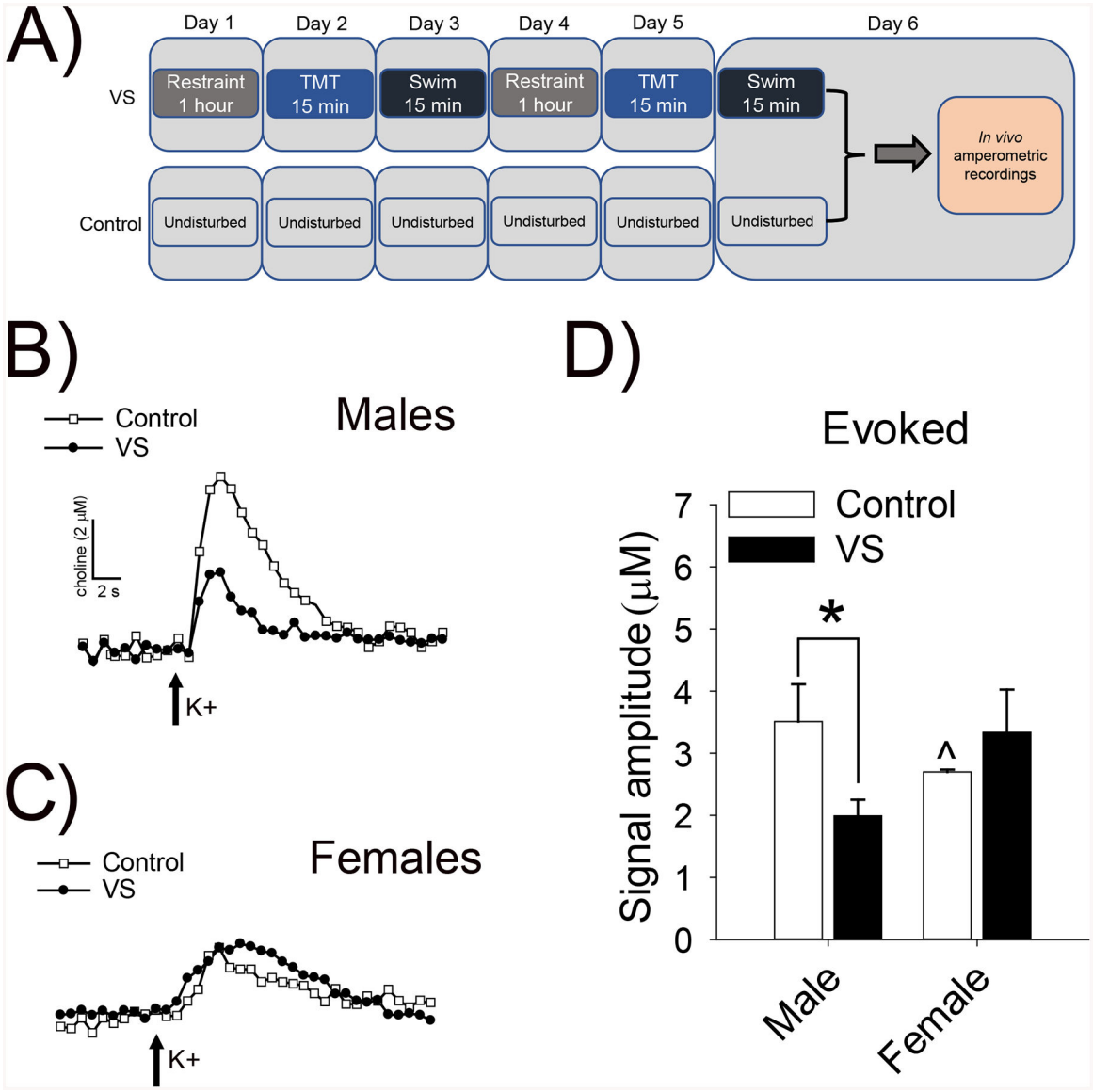

Figure 3. VS reduced ACh release in the mPFC of males.

Timelines depict the typical timing for recordings following VS and control manipulations (A). Representative traces illustrating choline spikes following brief depolarizing pulses of potassium in the mPFC of control and VS exposed male (B) and female (C) Sprague-Dawley rats. Choline signals reflect the hydrolysis of newly-released ACh from presynaptic cholinergic terminals. VS reduced ACh release in male rats, but did not alter ACh release in female rats (D). Asterisk indicates $p < .05$ from the same sex control group and the caret indicates a sex difference between male and female control rats.

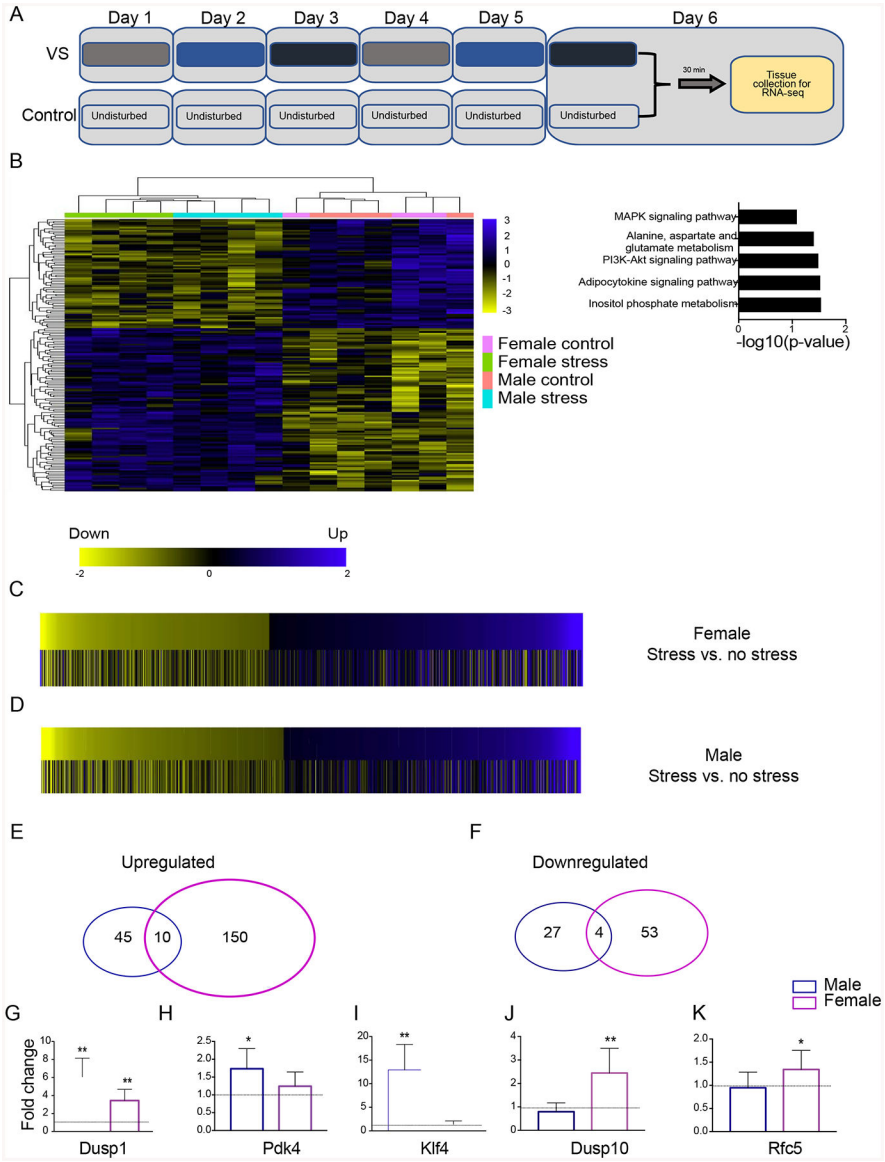

Figure 4. VS alters gene transcription in the NBM.

Timelines depict the timing for tissue collection following VS and control manipulations (A). Heatmap of genes differentially regulated by VS in Long Evans rats, with the counting reads of each gene plotted (B, left). KEGG pathway analysis on differentially expressed genes regulated by VS in both sexes (B, right). Heatmap of genes (with the log2FC plotted) differentially regulated by VS in females compared with the same genes in males (C), such that yellow bars indicate a negative log2FC (decreased gene expression by VS), black bars indicate no change by VS, and blue bars indicate a positive log2FC (increased gene expression by VS). Heatmap of genes (log2FC was plotted) differentially regulated by stress in males compared with females (D). Venn diagrams of total number of significantly upregulated genes (E) and downregulated genes (F) by VS in males (blue circle) and females (purple circle). Validation with qPCR in a different cohort of rats of the top 3 genes upregulated by VS in males: *Dusp1* (G), *Pdk4* (H), *Klf4* (I); and females: *Dusp1* (G),

Dusp10 (J), *Rfc5* (K). Notably, *Dusp1* was the top gene upregulated in both sexes and the other genes were regulated by VS in a sex-specific manner.

KEY RESOURCES TABLE

Resource Type	Specific Reagent or Resource	Source or Reference	Identifiers	Additional Information
Add additional rows as needed for each resource type	Include species and sex when applicable.	Include name of manufacturer, company, repository, individual, or research lab. Include PMID or DOI for references; use “this paper” if new.	Include catalog numbers, stock numbers, database IDs or accession numbers, and/or RRIDs. RRIDs are highly encouraged; search for RRIDs at https://scicrunch.org/resources.	Include any additional information or notes if necessary.
Antibody	mouse anti-ChAT monoclonal antibody	Millipore	Cat # MAB305	
Bacterial or Viral Strain	AAV9.CAG.Flex.eGFP.WPRE.bGH	University of Pennsylvania, Viral Vector Core	N/A	
Biological Sample				
Cell Line				
Chemical Compound or Drug	2,4,5-Trimethylthiazole	Sigma Aldrich	Cat # W332518–25G-K	
Commercial Assay Or Kit				
Deposited Data; Public Database				
Genetic Reagent				
Organism/Strain	rat: Long Evans-Tg(ChAT-Cre)5.1Deis, male and female	Rat Resource and Research Center	RRID:RGD_10401204	
Organism/Strain	rat: Sprague Dawley, male and female	Charles River	RRID:RGD_10395233	
Organism/Strain	rat: Long-Evans, male and female	Charles River	RRID:RGD_2308852	
Peptide, Recombinant Protein	Choline oxidase from Alcaligenes Sp. (E.C.1.1.3.17)	Millipore Sigma	Cat # C5896	
Recombinant DNA				
Sequence-Based Reagent				
Software; Algorithm	Neurolucida	MBF Bioscience Inc.	RRID:SCR_001775	
Software; Algorithm	FAST-16 data acquisition	Quanteon	N/A	
Transfected Construct				
Commercial Assay Or Kit	iScript cDNA Synthesis Kit	Bio-Rad	1708891BUN	
Commercial Assay Or Kit	RNeasy Mini Kit	Qiagen	74106	
Commercial Assay Or Kit	Universal RNA-Seq Library Preparation Kit	TECAN	Part No. 7102	
Sequence-Based Reagent	Primers for RT-qPCR, see Supplement methods	This paper	N/A	
Software; Algorithm	STAR	PMID: 23104886	STAR/2.5.2a	
Software; Algorithm	HTSeq Python package	PMID: 25260700	HTseq-count	

Resource Type	Specific Reagent or Resource	Source or Reference	Identifiers	Additional Information
Software; Algorithm	DESeq	PMID: 20979621		
Deposited data	GSE147806	NCBI GEO DataSets	https://www.ncbi.nlm.nih.gov/gds	
Software; Algorithm	samtools	PMID: 19505943	samtools-1.1	
Software; Algorithm	bedtools	PMID: 25199790		
Other				