EXPERIENCE-DEPENDENT NEUROPLASTICITY ACROSS THE LIFESPAN: FROM RISK TO RESILIENCE

EDITED BY: Erica R. Glasper and Gretchen N. Neigh

PUBLISHED IN: Frontiers in Behavioral Neuroscience

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88945-782-3
DOI 10.3389/978-2-88945-782-3

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

EXPERIENCE-DEPENDENT NEUROPLASTICITY ACROSS THE LIFESPAN: FROM RISK TO RESILIENCE

Topic Editors:
Erica R. Glasper, University of Maryland, United States
Gretchen N. Neigh, Virginia Commonwealth University, United States

Risk and resilience determine the impact of environmental exposures on outcomes from both physical and psychological challenges. This collection considers the factors which dictate risk and resilience and both the endogenous and environmental influences that can set the tone for responses to later life exposures.

Citation: Glasper, E. R., Neigh, G. N., eds. (2019). Experience-Dependent Neuroplasticity Across the Lifespan: From Risk to Resilience. Lausanne: Frontiers Media. doi: 10.3389/978-2-88945-782-3

Table of Contents

frontiers in Behavioral Neuroscience

EDITORIAL
published: 18 January 2019
doi: 10.3389/fnbeh.2018.00335

Editorial: Experience-Dependent Neuroplasticity Across the Lifespan: From Risk to Resilience

Erica R. Glasper[1]* *and Gretchen N. Neigh*[2]

[1] Department of Psychology, University of Maryland, College Park, MD, United States, [2] Department of Anatomy and Neurobiology, Virginia Commonwealth University, Richmond, VA, United States

Keywords: risk, resilience, neuroplasticity, experience, development

Editorial on the Research Topic

Experience-Dependent Neuroplasticity Across the Lifespan: From Risk to Resilience

Throughout life, experiences can profoundly shape the structure and function of the brain. This experience-dependent plasticity is observed in numerous cell types, brain regions, and circuits and can contribute significantly to stress regulation, mood, cognition, addiction, etc. This e-book highlights recent efforts in identifying experiences that may confer resilience to or pose a risk for the development of neuropsychiatric disorders and associated alterations in structural plasticity within the brain.

The importance of risk and resilience to long-term sequela of perturbations has been previously reviewed with regard to synaptic plasticity (Hyer et al., 2018), immune function (Bekhbat and Neigh, 2018a,b), and stress responsivity (Bourke et al., 2012; Bekhbat et al., 2017). The mechanisms which underlie the manifestation of risk and resilience following stressor exposure are not fully defined, but much progress has been made in individual disease states and conditions (Nemeth et al., 2014, 2015; Hodes et al., 2016; Neigh and Ali, 2016; Valdez et al., 2016). The mini-review in this e-book by Mukhara et al. focuses on the progress that has been made in terms of identification of candidate molecular mediators in the context of addiction and sets the framework for potential mechanistic studies. In addition to the importance of glucocorticoids and the dopaminergic system in the generation of risk and resilience, inflammatory-sensitive mechanisms within the brain have been identified as a key area of importance in the study of risk and resilience. The review by Finnell and Wood examines risk and resilience in the context of depression, and the authors further highlight the role of individual differences, first introduced in this e-book by Murthy and Gould, in the potential manifestation of risk vs. resilience with a focus on age, sex, and coping strategies. Further, the authors review mechanisms by which inflammatory cytokines and chemokines can alter function of neurons and glial cells precipitating changes in behavior.

Importantly, mechanistic drivers and the relative factors that produce risk and resilience are sensitive to developmental timing and level of exposure. The original research reports highlighted in this e-book span the developmental timeline and guide future inquiry into viable means by which to mitigate risk and produce resilience.

Experiences with offspring greatly influence the parental brain (Leuner et al., 2010), while parental care also influences offspring developmental (Rilling and Young, 2014; Bales and Saltzman, 2016). Traditional parenting-related plasticity is studied in the context of the dam and how interactions with offspring shape their risk or resilience throughout life. Using the biparental California mouse (*Peromyscus californicus*), Yohn et al. demonstrate that neuropeptide levels in social areas of the brain, and gonadal steroid hormones in females, are influenced by the amount of care provided by the father. These data contribute to a growing body of literature that suggests

Edited and reviewed by:
Nuno Sousa,
University of Minho, Portugal

***Correspondence:**
Erica R. Glasper
eglasper@umd.edu

Received: *17 December 2018*
Accepted: *20 December 2018*
Published: *18 January 2019*

Citation:
Glasper ER and Neigh GN (2019) Editorial: Experience-Dependent Neuroplasticity Across the Lifespan: From Risk to Resilience. Front. Behav. Neurosci. 12:335. doi: 10.3389/fnbeh.2018.00335

social behaviors, like paternal care, can program the developing brain via lasting effects on the neuroendocrine system.

Conversely, when paternal care is necessary for offspring survival and typical development, the lack of paternal care may increase susceptibility to mood disorders-similar to maternal models of early-life stress (Chen and Baram, 2016). Using paternal deprivation as a model of early-life adversity, Glasper et al. examined hippocampal plasticity of California mice during adulthood. Lack of paternal care increased anxiety-like behavior and behavioral despair in male and female offspring, however, cell survival of adult born cells in the dentate gyrus of the hippocampus was only reduced in paternally-deprived females. This apparent sex-difference in hippocampal structural plasticity following paternal deprivation contributes to our understanding of early-life stress reprogramming of neural regions involved in emotion (Chen and Baram, 2016) in a novel way, and along with Yohn et al. adds to the growing literature on sex differences in neural responsiveness to paternal care.

Adolescence is a time of great change in terms of the structure and function of the nervous system, including decreased cell proliferation and adult neurogenesis in the dentate gyrus of the hippocampus. Adolescence may increase susceptibility to stress-related perturbations during this time of vast neurobiological change. Shome et al. demonstrate that chronic treatment with exogenous glucocorticoids confers sex-specific effects on dentate gyrus structural plasticity. Specifically, chronic corticosterone treatment does not alter cell genesis or cell survival in females, and the effects in males are limited to immature neurons. This work suggests that adolescence-induced reductions in cell genesis may increase resiliency at a time of great environmental perturbations in a sex-specific way.

Medical experiences and somatic illnesses can further contribute to the manifestation of individual differences in susceptibility to stress-related behaviors. Caulfield et al. demonstrate the power of developmental asthma to alter both lung function and induce profound and enduring changes in the stress response system including altered gene expression in the brain and changes in stress-related behaviors. Further, this work demonstrates that individual differences prior to the manifestation of developmental asthma influence the long-term effects of developmental asthma and empirically highlight the important role of individual differences introduced by Murthy and Gould and Finnell and Wood.

Finally, environmental exposures are an important source of individual variability that can drive the manifestation of later-life somatic and mental health conditions. To this end, Pistoia et al. demonstrate the powerful influence of being exposed to the traumatic stress of a substantial earthquake during the adolescent period. Individuals from earthquake-affected areas exhibited an increase in anxiety and increased anticipation of threats including a more vigilant awareness of facial expressions. This inherent pattern of individual difference created in those from earthquake-affected areas could drive a susceptibility to future insults.

Collectively, the work presented in this e-book demonstrates that the manifestation of and mechanisms by which individuals respond to environmental stimuli, ranging from somatic conditions to environmental stressors, is shaped by experiences across the lifespan. These experience-induced changes shape the neural and somatic response to new challenges and exposures. This collection demonstrates that it is essential to consider the collective experiences and exposures of an organism when trying to predict risk vs. resilience. Furthermore, the salient effects of environmental exposures on lasting neural and somatic substrates should be considered when working to treat somatic and neuropsychiatric disorders.

AUTHOR CONTRIBUTIONS

EG and GN contributed to the conceptualization of the research topic and to the writing of this editorial.

REFERENCES

Bales, K. L., and Saltzman, W. (2016). Fathering in rodents: neurobiological substrates and consequences for offspring. *Horm. Behav.* 77, 249–259. doi: 10.1016/j.yhbeh.2015.05.021

Bekhbat, M., and Neigh, G. N. (2018a). Sex differences in the neuro-immune consequences of stress: focus on depression and anxiety. *Brain. Behav. Immun.* 67, 1–12. doi: 10.1016/j.bbi.2017.02.006

Bekhbat, M., and Neigh, G. N. (2018b). Stress-induced neuroimmune priming in males and females: comparable but not identical. *Brain. Behav. Immun.* 73, 149–150. doi: 10.1016/j.bbi.2018.05.001

Bekhbat, M., Rowson, S. A., and Neigh, G. N. (2017). Checks and balances: the glucocorticoid receptor and NFκB in good times and bad. *Front. Neuroendocrinol.* 46, 15–31. doi: 10.1016/j.yfrne.2017.05.001

Bourke, C. H., Harrell, C. S., and Neigh, G. N. (2012). Stress-induced sex differences: adaptations mediated by the glucocorticoid receptor. *Horm. Behav.* 62, 210–218. doi: 10.1016/j.yhbeh.2012.02.024

Chen, Y., and Baram, T. Z. (2016). Toward understanding how early-life stress reprograms cognitive and emotional brain networks. *Neuropsychopharmacology* 41, 197–206. doi: 10.1038/npp.2015.181

Hodes, R. J., Sierra, F., Austad, S. N., Epel, E., Neigh, G. N., Erlandson, K. M., et al. (2016). Disease drivers of aging. *Ann. N. Y. Acad. Sci.* 1386, 45–68. doi: 10.1111/nyas.13299

Hyer, M. M., Phillips, L. L., and Neigh, G. N. (2018). Sex differences in synaptic plasticity: hormones and beyond. *Front. Mol. Neurosci.* 11:266. doi: 10.3389/fnmol.2018.00266

Leuner, B., Glasper, E. R., and Gould, E. (2010). Parenting and plasticity. *Trends Neurosci.* 33, 465–473. doi: 10.1016/j.tins.2010.07.003

Neigh, G. N., and Ali, F. F. (2016). Co-morbidity of PTSD and immune system dysfunction: opportunities for treatment. *Curr. Opin. Pharmacol.* 29, 104–110. doi: 10.1016/j.coph.2016.07.011

Nemeth, C. L., Bekhbat, M., and Neigh, G. N. (2015). Neural effects of inflammation, cardiovascular disease, and HIV: parallel, perpendicular, or progressive? *Neuroscience* 302, 165–173. doi: 10.1016/j.neuroscience.2014.09.016

Nemeth, C. L., Haroon, E., and Neigh, G. N. (2014). Heartsick: psychiatric and inflammatory implications of cerebromicrovascular disease. *Int. J. Geriatr. Psychiatry* 29, 577–85. doi: 10.1002/gps.4046

Rilling, J. K., and Young, L. J. (2014). The biology of mammalian parenting and its effect on offspring social development. *Science* 345, 771–776. doi: 10.1126/science.1252723

Valdez, A. N., Rubin, L. H., and Neigh, G. N. (2016). Untangling the Gordian knot of HIV, stress, and cognitive impairment. *Neurobiol. Stress* 4, 44–54. doi: 10.1016/j.ynstr.2016.02.005

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Behavioral Neuroscience

OPINION
published: 31 July 2018
doi: 10.3389/fnbeh.2018.00157

Early Life Stress in Rodents: Animal Models of Illness or Resilience?

*Sahana Murthy and Elizabeth Gould**

Princeton Neuroscience Institute, Princeton University, Princeton, NJ, United States

Keywords: early life stress, anxiety, depression, rodent models, resilience

Early life adversity is a known risk factor for mood and anxiety disorders in adult humans (Heim et al., 2010; Huh et al., 2014; Rehan et al., 2017). Given the prevalence of both maltreatment in childhood and mental illness in adulthood, understanding the neurobiological mechanisms of this connection is important as it may suggest targets for new therapeutic interventions. Ethical constraints on conducting studies with humans have highlighted the need for reliable and robust animal models that researchers can utilize to identify relevant neurobiological processes (Guzman et al., 2016). Since the work of Harlow and colleagues beginning in the 1940s, which involved raising infant macaques with cloth and wire mothers (reviewed in van der Horst and van der Veer, 2008), researchers have sought to develop useful animal models of early life adversity. These and other more recent studies have shown obvious behavioral abnormalities in monkeys subjected to early life stress (ELS) (Schino et al., 2001; Corcoran et al., 2012; Howell et al., 2014). Despite the relevance of these models to humans, nonhuman primates have practical and ethical limitations that are obstacles for their use in high-throughput studies. By contrast, animal models of early life stress in rodents, which were first used in the laboratory more than 50 years ago (Levine, 1957), have gained in usage.

One of the most commonly used manipulations to produce a rodent model of ELS has been maternal separation. Studies have shown that maternal separation in rats, as long as it is of sufficient duration (typically 3 h/day during the first 2 postnatal weeks of life) increases anxiety- and depressive-like behaviors in adulthood, suggesting that it has translational validity (Janus, 1987; Huot et al., 2001; Kalinichev et al., 2002; Romeo et al., 2003; Daniels et al., 2004; Lee et al., 2007; Wei et al., 2010; Masrour et al., 2018). However, other studies in both rats and mice have shown considerable variability in behavioral results from maternal separation, with several reports showing no behavioral effect (Lehmann et al., 1999; Eklund and Arborelius, 2006; Slotten et al., 2006; Millstein and Holmes, 2007; Savignac et al., 2011). In addition to inconsistent behavioral findings with this model, concerns have been raised about whether maternal separation mimics neglect, abuse or a combination of both. It has been reported that after prolonged separation, maternal behavior toward pups differs and these differences may be as important, if not more, than the lack of contact with the mother (Boccia and Pedersen, 2001; Huot et al., 2004). Some reports have also observed that dams increase maternal care post-separation possibly attenuating the effects of the separation itself (Millstein and Holmes, 2007). The type of human maltreatment that rodent maternal separation reflects might be important for establishing its translational validity, since human studies have separated early adverse experiences into several categories, including emotional abuse, emotional neglect, physical abuse, physical neglect and sexual abuse (Kendler et al., 2004; van Harmelen et al., 2010; Young and Widom, 2014; Rehan et al., 2017; Gallo et al., 2018) and some studies suggest that the type of maltreatment may be important for the adult outcome in terms of behavioral dysfunction (Huh et al., 2014; Young and Widom, 2014).

To address concerns about the unspecified nature of the maternal separation manipulation, researchers have developed another way to impair maternal care with the limited bedding/nesting model (Brunson et al., 2005; Cui et al., 2006; Ivy et al., 2008; Rice et al., 2008). The most extreme version of this model involves housing dams in a wire mesh floored cage with no bedding and a

OPEN ACCESS

Edited by:
Xiao-Dong Wang, Zhejiang University, China

Reviewed by:
Brittany Rollins Howell, University of Minnesota Twin Cities, United States

****Correspondence:***
Elizabeth Gould
goulde@princeton.edu

Received: *08 May 2018*
Accepted: *06 July 2018*
Published: *31 July 2018*

Citation:
Murthy S and Gould E (2018) Early Life Stress in Rodents: Animal Models of Illness or Resilience? Front. Behav. Neurosci. 12:157. doi: 10.3389/fnbeh.2018.00157

scarcity of material with which to make a nest, while variations involve just limiting nesting material (Walker et al., 2017). The result is an increase in maternal anxiety and fractured caregiving where behavior toward the pups might be interpreted as abusive (Rice et al., 2008). As with the earlier investigations of the maternal separation model, some studies using this manipulation reported evidence for increased anxiety- and depressive-like behavior in adulthood (Cui et al., 2006; Dalle Molle et al., 2012; Raineki et al., 2012; Wang et al., 2012), supporting its translational validity. However, other studies using this model failed to find an increase in anxiety- or depressive-like behavior (Brunson et al., 2005; Rice et al., 2008; van der Kooij et al., 2015; Johnson et al., 2018) raising questions about reliability similar to those observed with the maternal separation model.

Contradictory results of studies using both of these rodent models are puzzling and may be attributable to differences in experimental design. To fully understand these discrepancies, many factors must be considered (**Figure 1**). First, the genetic background of the experimental animal is important. Human studies have clearly shown genetic predisposition to mood and anxiety disorders and it follows that this factor should be considered in studies using experimental animals to model the human condition. Studies have shown varying effects of maternal separation on anxiety- and depressive-like behaviors in different strains of mice; the C57Bl/6 strain appears to be most resistant to stress compared to other strains, such as the Balb/c strain, which is inherently more anxious (Millstein and Holmes, 2007; Wei et al., 2010; Savignac et al., 2011). However, different studies using the same strain have reported conflicting results with seemingly identical ELS manipulations, so genetic strain differences cannot account for all of the variance in the literature. It should be noted, however, that individual subtler genetic differences within a specific rodent strain may be relevant to establishing vulnerability to such manipulations. That is, ELS manipulations likely impact some animals more than others and such variability may obscure overall group differences in behavior. Second, the sex of the animal should be considered. Somewhat paradoxically given that women exhibit greater prevalence of mood/anxiety disorders than do men (Altemus et al., 2014), several rodent studies have shown that ELS produces either no effect or a reduction in anxiety- and depressive-like behaviors in females (Lehmann et al., 1999; McIntosh et al., 1999; Eklund and Arborelius, 2006; Slotten et al., 2006). These unexpected results raise questions about whether the standard laboratory tests of anxiety- and depressive-like behavior, which were developed for use in males and typically involve measures of behavioral inhibition, are accurate measures of these states in female rodents, given known estrous cycle variations in behavioral activity levels. Clearly, the field would benefit from new sensitive behavioral assays that are useful for both sexes, particularly given the need to correct the under-emphasis of research on females (Clayton and Collins, 2014).

The timing and duration of the stressful experience during the postnatal period may also be important to consider. In rodent studies, differential effects of early vs. late postnatal stress exposure on depressive-like behaviors have been demonstrated (van der Kooij et al., 2015; Peña et al., 2017). By contrast, however, a recent human study concluded that data on the link between childhood maltreatment and psychopathology do not fit a sensitive period theoretical model (Dunn et al., 2018), again raising questions about the direct translational validity of

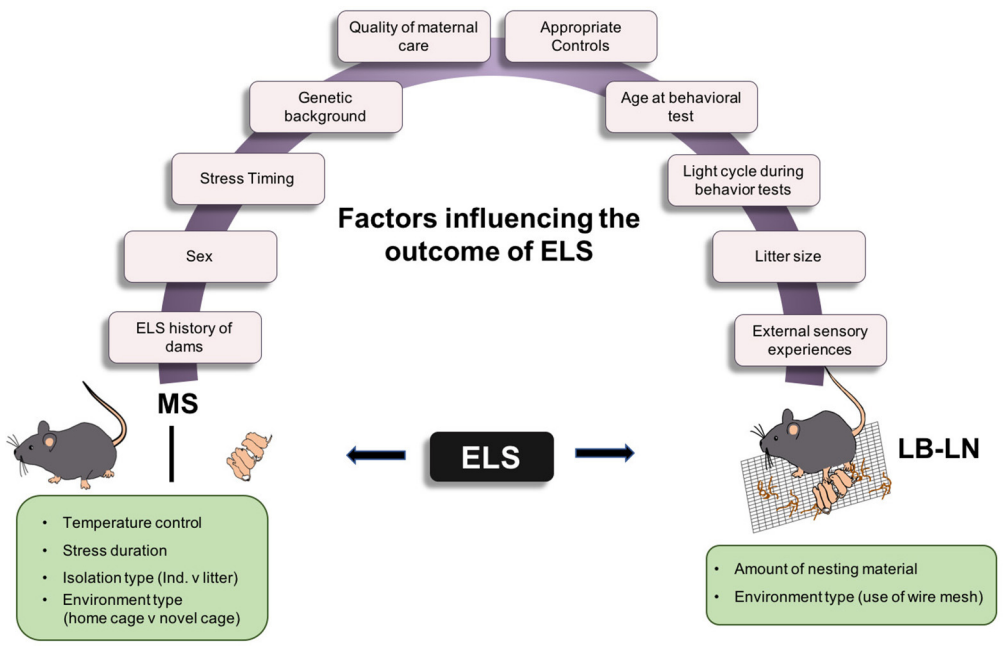

FIGURE 1 | Factors influencing variability in ELS animal models. Cartoon depicting the two most commonly used models of ELS and the different factors influencing behavioral outcomes in adulthood. MS, Maternal separation stress; LB-LN, Limited bedding-Limited nesting.

some ELS models in rodents. It is likely relevant that the HPA axis response to stress is attenuated in pups during the stress hyporesponsive period, a phenomenon that serves a protective effect on the developing brain (Sapolsky and Meaney, 1986). A similar state has been reported in humans up until about 1 year of age, but it does not extend throughout childhood when the majority of reported maltreatment occurs (Gunnar and Donzella, 2002).

The duration of stress seems to be more definitively associated with worse outcomes compared to the timing of stress, and data from human studies support a cumulative and/or recency model of stress effects on vulnerability to psychopathology (Dunn et al., 2018). To address the issue about the duration of stress as well as inconsistencies in the ELS literature, researchers have developed "two-hit" models that incorporate maternal separation followed by additional stress, either shortly thereafter or in adulthood. The models are based on the assumption that the first stressful period may create an internal vulnerability that is alone insufficient to manifest itself behaviorally, but when aggravated by subsequent stress, produces detectable behavioral changes. One set of such studies used longer periods of separation followed by early weaning of pups (George et al., 2010). Early weaning by itself has been shown to increase anxiety-like behavior in adulthood (Kikusui et al., 2004) and when combined with maternal separation, it not only increases anxiety-like behaviors but also results in hyperactivity, gene dysregulation and neuroanatomical changes to the brain; some of which have been observed in humans with a history of early life abuse. Another set of such studies used maternal separation and/or limited bedding followed by exposure to chronic stress in adulthood (Vargas et al., 2016; Peña et al., 2017). Both of these approaches mimic the "dose-response" or "cumulative" stress links to mental illness that have been described in humans. However, like the other rodent models of ELS, data from these two-hit models need to be interpreted with caution as null effects have also been reported (Santarelli et al., 2017; Tan et al., 2017).

Notwithstanding the potential importance of strain, sex, timing, duration, type of stress experience and other factors (**Figure 1**) across studies as reasons for variable results, it is clear that variable results can emerge even in the face of virtually identical experimental designs. What is the explanation for these differences? While we do not know for certain, there are some important points to consider. First, baseline housing and testing conditions may vary across laboratories in seemingly unspecified ways (Cavigelli et al., 2006; Sorge et al., 2014), adding additional stress to both control and experimental groups and potentially reducing the behavioral differences between them. Second, evidence suggests that rodent maternal behavior varies considerably even within control groups (Francis and Meaney, 1999). In other words, some rat and mouse dams may be more capable of compensating for the effects of maternal separation or limited bedding than others. This could be influenced by the early life experiences of the dams themselves and the amount of stress they were exposed to before entering breeding. This natural variation in maternal behavior may introduce additional variability into ELS-induced long-term behavioral outcomes. Third, perhaps related to the second point, rodent populations likely display considerable individual variability in response to ELS, such that depending on the cohort examined, statistically significant differences in anxiety- and depressive-like behavior may or may not be detectable. Thus, reproducible significant differences may require larger numbers of animals than are often used in such studies, consistent with what has been the norm for human studies (Collins and Tabak, 2014). In addition, these studies might be more informative if the data from rodents subjected to ELS manipulations were analyzed in ways that do not group them together with the assumption that they comprise a homogenous group. In searching for neurobiological mechanisms underlying behavioral signs of mental illness, it may be more fruitful to separate out the experimental animals that show robust ELS-induced increases in anxiety- and depressive-like behavior. This approach might reveal informative correlations between brain changes and relevant behaviors. While this suggestion makes experimental designs and statistical analyses more complicated than commonly used methods of comparing means between groups, it may produce more reliable results across laboratories.

Considering rodent populations as heterogeneous with regard to their susceptibility to ELS-induced behavioral changes would address an interesting parallel with humans. While the connection between early life adversity and mood/anxiety disorders in humans has been widely accepted, it is perhaps less well-known that the majority of people subjected to childhood maltreatment (>70%) do not show anxiety and depression symptoms that are clinically significant (Rehan et al., 2017). Thus, as with rodents, humans display a considerable amount of resilience and resistance to early life adversity, a phenomenon that deserves scientific attention as it may provide clues about how to encourage these characteristics in the entire population. Finally, it deserves mention that many people develop anxiety and mood disorders that are not retrospectively traceable to childhood maltreatment, so examining control rodents that score as more anxious/depressed despite a lack of prior stress manipulation may be informative as well. Here again, looking at individual differences within groups may be most informative and also help to reduce the inconsistency across studies using rodent models of stress-induced mental illness.

AUTHOR CONTRIBUTIONS

SM wrote the article, edited the article and made the figure. EG wrote the article, edited the article and edited the figure.

FUNDING

This work was supported by grant NIH MH117459-01 (EG) and a CV Starr Fellowship (SM).

REFERENCES

Altemus, M., Sarvaiya, N., and Neill Epperson, C. (2014). Sex differences in anxiety and depression clinical perspectives. *Front. Neuroendocrinol.* 35, 320–330. doi: 10.1016/j.yfrne.2014.05.004

Boccia, M. L., and Pedersen, C. A. (2001). Brief vs. long maternal separations in infancy: contrasting relationships with adult maternal behavior and lactation levels of aggression and anxiety. *Psychoneuroendocrinology* 26, 657–672. doi: 10.1016/S0306-4530(01)00019-1

Brunson, K. L., Kramár, E., Lin, B., Chen, Y., Colgin, L. L., Yanagihara, T. K., et al. (2005). Mechanisms of late-onset cognitive decline after early-life stress. *J. Neurosci.* 25, 9328–9338. doi: 10.1523/JNEUROSCI.2281-05.2005

Cavigelli, S. A., Guhad, F. A., Ceballos, R. M., Whetzel, C. A., Nevalainen, T., Lang, C. M., et al. (2006). Fecal corticoid metabolites in aged male and female rats after husbandry-related disturbances in the colony room. *J. Am. Assoc. Lab Anim. Sci.* 45, 17–21. doi: 10.1530/EC-17-0338

Clayton, J. A., and Collins, F. S. (2014). Policy: NIH to balance sex in cell and animal studies. *Nature* 509, 282–283. doi: 10.1038/509282a

Collins, F. S., and Tabak, L. A. (2014). Policy: NIH plans to enhance reproducibility. *Nature* 505, 612–613. doi: 10.1038/505612a

Corcoran, C. A., Pierre, P. J., Haddad, T., Bice, C., Suomi, S. J., Grant, K. A., et al. (2012). Long-term effects of differential early rearing in rhesus macaques: behavioral reactivity in adulthood. *Dev. Psychobiol.* 54, 546–555. doi: 10.1002/dev.20613

Cui, M., Yang, Y., Yang, J., Zhang, J., Han, H., Ma, W., et al. (2006). Enriched environment experience overcomes the memory deficits and depressive-like behavior induced by early life stress. *Neurosci. Lett.* 404, 208–212. doi: 10.1016/j.neulet.2006.05.048

Dalle Molle, R., Portella, A. K., Goldani, M. Z., Kapczinski, F. P., Leistner-Segal, S., Salum, G. A., et al. (2012). Associations between parenting behavior and anxiety in a rodent model and a clinical sample: relationship to peripheral BDNF levels. *Trans. Psychiatry* 2:e195. doi: 10.1038/tp.2012.126

Daniels, W. M., Pietersen, C. Y., Carstens, M. E., and Stein, D. J. (2004). Maternal separation in rats leads to anxiety-like behavior and a blunted ACTH response and altered neurotransmitter levels in response to a subsequent stressor. *Metab. Brain Dis.* 19, 3–14. doi: 10.1023/B:MEBR.0000027412.19664.b3

Dunn, E. C., Soare, T. W., Raffeld, M. R., Busso, D. S., Crawford, K. M., Davis, K. A., et al. (2018). What life course theoretical models best explain the relationship between exposure to childhood adversity and psychopathology symptoms: recency, accumulation, or sensitive periods? *Psychol. Med.* 26, 1–11. doi: 10.1017/S0033291718000181

Eklund, M. B., and Arborelius, L. (2006). Twice daily long maternal separations in Wistar rats decreases anxiety-like behaviour in females but does not affect males. *Behav. Brain Res.* 172, 278–285. doi: 10.1016/j.bbr.2006.05.015

Francis, D. D., and Meaney, M. J. (1999). Maternal care and the development of stress responses. *Curr. Opin. Neurobiol.* 9, 128–134. doi: 10.1016/S0959-4388(99)80016-6

Gallo, E. A. G., Munhoz, T. N., Loret de Mola, C., and Murray, J. (2018). Gender differences in the effects of childhood maltreatment on adult depression and anxiety: a systematic review and meta-analysis. *Child Abuse Negl.* 79, 107–114. doi: 10.1016/j.chiabu.2018.01.003

George, E. D., Bordner, K. A., Elwafi, H. M., and Simen, A. A. (2010). Maternal separation with early weaning: a novel mouse model of early life neglect. *BMC Neurosci.* 11:123. doi: 10.1186/1471-2202-11-123

Gunnar, M. R., and Donzella, B. (2002). Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology* 27, 199–220. doi: 10.1016/S0306-4530(01)00045-2

Guzman, D. B., Howell, B., and Sanchez, M. (2016). "Early life stress and development: preclinical science," in *Posttraumatic Stress Disorder: From Neurobiology to Treatment,* ed J. D. Bremner (Hoboken, NJ: Wiley-Blackwell), 61–80.

Heim, C., Shugart, M., Craighead, W. E., and Nemeroff, C. B. (2010). Neurobiological and psychiatric consequences of child abuse and neglect. *Dev. Psychobiol.* 52, 671–690. doi: 10.1002/dev.20494

Howell, B. R., Grand, A. P., McCormack, K. M., Shi, Y., LaPrarie, J. L., Maestripieri, D., et al. (2014). Early adverse experience increases emotional reactivity in juvenile rhesus macaques: relation to amygdala volume. *Dev. Psychobiol.* 56, 1735–1746. doi: 10.1002/dev.21237

Huh, H. J., Kim, S. Y., Yu, J. J., and Chae, J. H. (2014). Childhood trauma and adult interpersonal relationship problems in patients with depression and anxiety disorders. *Ann. Gen. Psychiatry* 13:26. doi: 10.1186/s12991-014-0026-y

Huot, R. L., Gonzalez, M. E., Ladd, C. O., Thrivikraman, K. V., and Plotsky, P. M. (2004). Foster litters prevent hypothalamic-pituitary-adrenal axis sensitization mediated by neonatal maternal separation. *Psychoneuroendocrinology* 29, 279–289. doi: 10.1016/S0306-4530(03)00028-3

Huot, R. L., Thrivikraman, K. V., Meaney, M. J., and Plotsky, P. M. (2001). Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in Long Evans rats and reversal with antidepressant treatment. *Psychopharmacology* 158, 366–373. doi: 10.1007/s002130100701

Ivy, A. S., Brunson, K. L., Sandman, C., and Baram, T. Z. (2008). Dysfunctional nurturing behavior in rat dams with limited access to nesting material: a clinically relevant model for early-life stress. *Neuroscience* 154, 1132–1142. doi: 10.1016/j.neuroscience.2008.04.019

Janus, K. (1987). Effects of early separation of young rats from the mother on their open-field behavior. *Physiol. Behav.* 40, 711–715. doi: 10.1016/0031-9384(87)90272-1

Johnson, F. K., Delpech, J. C., Thompson, G. J., Wei, L., Hao, J., Herman, P., et al. (2018). Amygdala hyper-connectivity in a mouse model of unpredictable early life stress. *Transl. Psychiatry* 8:49. doi: 10.1038/s41398-018-0092-z

Kalinichev, M., Easterling, K. W., Plotsky, P. M., and Holtzman, S. G. (2002). Long-lasting changes in stress-induced corticosterone response and anxiety-like behaviors as a consequence of neonatal maternal separation in Long-Evans rats. *Pharmacol. Biochem. Behav.* 73, 131–140. doi: 10.1016/S0091-3057(02)00781-5

Kendler, K. S., Kuhn, J. W., and Prescott, C. A. (2004). Childhood sexual abuse, stressful life events and risk for major depression in women. *Psychol. Med.* 34, 1475–1482. doi: 10.1017/S003329170400265X

Kikusui T., Takeuchi Y., and Mori Y. (2004). Early weaning induces anxiety and aggression in adult mice. *Physiol. Behav.* 81, 37–42. doi: 10.1016/j.physbeh.2003.12.016

Lee, J. H., Kim, H. J., Kim, J. G., Ryu, V., Kim, B. T., Kang, D. W., et al. (2007). Depressive behaviors and decreased expression of serotonin reuptake transporter in rats that experienced neonatal maternal separation. *Neurosci. Res.* 58, 32–39. doi: 10.1016/j.neures.2007.01.008

Lehmann, J., Pryce, C. R., Bettschen, D., and Feldon, J. (1999). The maternal separation paradigm and adult emotionality and cognition in male and female Wistar rats. *Pharmacol. Biochem. Behav.* 64, 705–715. doi: 10.1016/S0091-3057(99)00150-1

Levine, S. (1957). Infantile experience and resistance to physiological stress. *Science* 126:405. doi: 10.1126/science.126.3270.405

Masrour, F. F., Peeri, M., Azarbayjani, M. A., and Hosseini, M. J. (2018). Voluntary exercise during adolescence mitigated negative the effects of maternal separation stress on the depressive-like behaviors of adult male rats: role of nmda receptors. *Neurochem. Res.* 43, 1067–1074. doi: 10.1007/s11064-018-2519-6

McIntosh, J., Anisman, H., and Merali, Z. (1999). Short- and long-periods of neonatal maternal separation differentially affect anxiety and feeding in adult rats: gender-dependent effects. *Dev. Brain Res.* 113, 97–106. doi: 10.1016/S0165-3806(99)00005-X

Millstein, R. A., and Holmes, A. (2007). Effects of repeated maternal separation on anxiety- and depression-related phenotypes in different mouse strains. *Neurosci. Biobehav. Rev.* 31, 3–17. doi: 10.1016/j.neubiorev.2006.05.003

Peña, C. J., Kronman, H. G., Walker, D. M., Cates, H. M., Bagot, R. C., Purushothaman, I., et al. (2017). Early life stress confers lifelong stress susceptibility in mice via ventral tegmental area OTX2. *Science* 356, 1185–1188. doi: 10.1126/science.aan4491

Raineki, C., Cortes, M. R., Belnoue, L., and Sullivan, R. M. (2012). Effects of early-life abuse differ across development: infant social behavior deficits are followed by adolescent depressive-like behaviors mediated by the amygdala. *J. Neurosci.* 32, 7758–7765. doi: 10.1523/JNEUROSCI.5843-11.2012

Rehan, W., Antfolk, J., Johansson, A., Jern, P., and Santtila, P. (2017). Experiences of severe childhood maltreatment, depression, anxiety and alcohol abuse among adults in Finland. *PLoS ONE* 12:e0177252. doi: 10.1371/journal.pone.0177252

Rice, C. J., Sandman, C. A., Lenjavi, M. R., and Baram, T. Z. (2008). A novel mouse model for acute and long-lasting consequences of early life stress. *Endocrinology* 149, 4892–4900. doi: 10.1210/en.2008-0633

Romeo, R. D., Mueller, A., Sisti, H. M., Ogawa, S., McEwen, B. S., and Brake, W. G. (2003). Anxiety and fear behaviors in adult male and female C57BL/6 mice are modulated by maternal separation. *Horm. Behav*. 43, 561–567. doi: 10.1016/S0018-506X(03)00063-1

Santarelli, S., Zimmermann, C., Kalideris, G., Lesuis, S. L., Arloth, J., Uribe, A., et al. (2017). An adverse early life environment can enhance stress resilience in adulthood. *Psychoneuroendocrinology* 78, 213–221. doi: 10.1016/j.psyneuen.2017.01.021

Sapolsky, R. M., and Meaney, M. J. (1986). Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. *Brain Res.* 396, 64–76. doi: 10.1016/0165-0173(86)90010-X

Savignac, H. M., Dinan, T. G., and Cryan, J. F. (2011). Resistance to early-life stress in mice: effects of genetic background and stress duration. *Front. Behav. Neurosci.* 5:13. doi: 10.3389/fnbeh.2011.00013

Schino, G., Speranza, L., and Troisi, A. (2001). Early maternal rejection and later social anxiety in juvenile and adult Japanese macaques. *Dev. Psychobiol.* 38, 186–190. doi: 10.1002/dev.1012

Slotten, H. A., Kalinichev, M., Hagan, J. J., Marsden, C. A., and Fone, K. C. (2006). Long-lasting changes in behavioural and neuroendocrine indices in the rat following neonatal maternal separation: gender-dependent effects. *Brain Res.* 1097, 123–132. doi: 10.1016/j.brainres.2006.04.066

Sorge, R. E., Martin, L. J., Isbester, K. A., Sotocinal, S. G., Rosen, S., Tuttle, A. H., et al. (2014). Olfactory exposure to males, including men, causes stress and related analgesia in rodents. *Nat. Methods* 11, 629–632. doi: 10.1038/nmeth.2935

Tan, S., Ho, H. S., Song, A. Y., Low, J., and Je, H. S. (2017). Maternal separation does not produce a significant behavioral change in mice. *Exp. Neurobiol.* 26, 390–398. doi: 10.5607/en.2017.26.6.390

van der Horst, F. C., and van der Veer, R. (2008). Loneliness in infancy: Harry Harlow, John Bowlby and issues of separation. *Integr. Psychol. Behav. Sci*. 42, 325–335. doi: 10.1007/s12124-008-9071-x

van der Kooij, M. A., Grosse, J., Zanoletti, O., Papilloud, A., and Sandi, C. (2015). The effects of stress during early postnatal periods on behavior and hippocampal neuroplasticity markers in adult male mice. *Neuroscience* 311, 508–518. doi: 10.1016/j.neuroscience.2015.10.058

van Harmelen, A. L., de Jong, P. J., Glashouwer, K. A., Spinhoven, P., Penninx, B. W., and Elzinga, B. M. (2010). Child abuse and negative explicit and automatic self-associations: the cognitive scars of emotional maltreatment. *Behav. Res. Ther*. 48, 486–494. doi: 10.1016/j.brat.2010.02.003

Vargas, J., Junco, M., Gomez, C., and Lajud, N. (2016). Early life stress increases metabolic risk, hpa axis reactivity, and depressive-like behavior when combined with postweaning social isolation in rats. *PLoS ONE* 11:e0162665. doi: 10.1371/journal.pone.0162665

Walker, C. D., Bath, K. G., Joels, M., Korosi, A., Larauche, M., Lucassen, P. J., et al. (2017). Chronic early life stress induced by limited bedding and nesting (LBN) material in rodents: critical considerations of methodology, outcomes and translational potential. *Stress* 20, 421–448. doi: 10.1080/10253890.2017.1343296

Wang, X. D., Labermaier, C., Holsboer, F., Wurst, W., Deussing, J. M., Muller, M. B., et al. (2012). Early-life stress-induced anxiety-related behavior in adult mice partially requires forebrain corticotropin-releasing hormone receptor 1. *Eur. J. Neurosci.* 36, 2360–2367. doi: 10.1111/j.1460-9568.2012.08148.x

Wei, L., David, A., Duman, R. S., Anisman, H., and Kaffman, A. (2010). Early life stress increases anxiety-like behavior in Balb c mice despite a compensatory increase in levels of postnatal maternal care. *Horm. Behav*. 57, 396–404. doi: 10.1016/j.yhbeh.2010.01.007

Young, J. C., and Widom, C. S. (2014). Long-term effects of child abuse and neglect on emotion processing in adulthood. *Child Abuse Negl.* 38, 1369–1381. doi: 10.1016/j.chiabu.2014.03.008

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Behavioral Neuroscience

MINI REVIEW
published: 21 December 2018
doi: 10.3389/fnbeh.2018.00309

Stress as a Risk Factor for Substance Use Disorders: A Mini-Review of Molecular Mediators

Deepika Mukhara[1], *Matthew L. Banks*[2] *and Gretchen N. Neigh*[1]*

[1]*Department of Anatomy & Neurobiology, Virginia Commonwealth University, Richmond, VA, United States,* [2]*Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA, United States*

The extant literature supports the role of stress in enhancing the susceptibility of drug abuse progressing to a substance use disorder diagnosis. However, the molecular mediators by which stress enhances the progression from cocaine abuse to cocaine use disorder *via* the mesolimbic pathway remain elusive. In this mini-review article, we highlight three mechanisms by which glucocorticoids (GCs) and the dopaminergic system interact. First, GCs upregulate tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine (DA) synthesis. Second, GCs downregulate monoamine-oxidase (MAO), an enzyme responsible for DA removal. Lastly, GCs are hypothesized to decrease DA reuptake, subsequently increasing synaptic DA. Based on these interactions, we review preclinical literature highlighting how stress modulates the mesolimbic pathway, including the ventral tegmental area (VTA) and nucleus accumbens (NAcs), to alter cocaine abuse-related effects. Taken together, stress enhances cocaine's abuse-related effects at multiple points along the VTA mesolimbic projection, and uniquely in the NAcs through a positive feedback type mechanism. Furthermore, we highlight future directions to elucidate the interaction between the prefrontal cortex (PFC) and key intermediaries including ΔFosB, cAMP response element binding protein (CREB) and cyclin-dependent kinase 5 (CDK5) to highlight possible mechanisms that underlie stress-induced acceleration of the progression to a cocaine use disorder diagnosis.

Keywords: stress, addiction, cocaine, VTA, NAc, drugs

OPEN ACCESS

Edited by:
James P. Herman,
University of Cincinnati, United States

Reviewed by:
Fabio Marti,
Institut National de la Santé et de la Recherche Médicale (INSERM), France
Susan Martelle,
University of Cincinnati, United States

***Correspondence:**
Gretchen N. Neigh
gretchen.mccandless@vcuhealth.org

Received: *28 June 2018*
Accepted: *28 November 2018*
Published: *21 December 2018*

Citation:
Mukhara D, Banks ML and Neigh GN (2018) Stress as a Risk Factor for Substance Use Disorders: A Mini-Review of Molecular Mediators. Front. Behav. Neurosci. 12:309. doi: 10.3389/fnbeh.2018.00309

INTRODUCTION

The Diagnostic and Statistical Manual of Mental Disorders (DSM)-V criteria for substance use disorders is defined as "recurrent use of alcohol and/or other drugs causes clinically and functionally significant impairment, such as health problems, disability and failure to meet major responsibilities at work, school, or home" (American Psychiatric Association, 2013). Substance use disorders may range from mild-to-severe and include a variety of substances such as opiates, nicotine, alcohol, cocaine and others, each of which has different mechanisms of action and protein targets. While cocaine exposure does not always progress to a cocaine use disorder diagnosis, a subset of individuals will progress to severe cocaine use disorder or what is referred to as cocaine "addiction" in the preclinical literature. Although epidemiological reports vary, cocaine use disorder is estimated to have an incidence of 0.1% worldwide (Shield et al., 2018). Although the factors that drive progression to substance use disorders are not fully defined, several lines of evidence

suggest stress exacerbates susceptibility to the abuse-related effects of drugs (Piazza and Le Moal, 1998; Sinha, 2001; Cleck and Blendy, 2008). For example, neonatal stress selectively enhances the acquisition of cocaine self-administration in rats, but does not augment self-administration when the reinforcer is food (Kosten et al., 2000). Social housing stress in nonhuman primates enhances the reinforcing effects of cocaine in subordinate monkeys (Morgan et al., 2002); however, early life stress produced by maternal separation does not enhance the abuse-related effects of cocaine in nonhuman primates (Ewing Corcoran and Howell, 2010).

Moreover, cumulative adversity is significantly predictive of drug abuse in a dose-dependent manner (Sinha, 2008). In fact, the limbic-hypothalamic-pituitary-adrenal axis (LHPA) axis, responsible for governing the stress response, has substantial overlap with the mesolimbic "reward" pathway involved in reward circuitry (Koob, 2009). The mesolimbic pathway involves dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAcs) and olfactory tubercle in the brain (Quintero, 2013). This pathway is hypothesized to have a critical role in the perception of pleasure and is conceptualized by Koob (2011) to have several key functions: associating meaning to reward-related cues, motivating goal-oriented behavior and general activation. In this mini-review article, we will focus on the impact of stress on cocaine abuse-related effects mediated through the mesolimbic dopamine (DA) "reward" pathway. Given the considerable evidence supporting an impact of stress on substance use disorder susceptibility and relapse, improved understanding of the mechanisms by which stress alters the abuse-related effects of drugs may provide insight into novel molecular targets for therapeutic interventions.

Underlying Mechanisms of Cocaine Abuse

Cocaine nonselectively binds to all three monoamine transporters (DA, norepinephrine, and serotonin) and prevents the reuptake of these monoamines into the presynaptic terminal thereby enhancing monoamine neurotransmission. Cocaine inhibition of the DA transporter is thought to be the primary mediator of the abuse-related effects of cocaine (Ritz et al., 1987; Volkow et al., 1997). Despite the DA transporter being the primary target for cocaine's abuse-related effects, repeated cocaine exposure does not alter presynaptic DA transporter availability in either humans (Wang et al., 1997) or nonhuman primates (Czoty et al., 2007). However, repeated cocaine exposure has been shown to increase serotonin and norepinephrine transporter densities in nonhuman primates (Macey et al., 2003; Beveridge et al., 2005; Banks et al., 2008). Furthermore, repeated cocaine exposure downregulates both presynaptic and postsynaptic DA receptors in humans (Volkow et al., 1990, 1993), nonhuman primates (Nader et al., 2006) and rats (Laurier et al., 1994). These cocaine-induced decreases in DA receptors on both pre- and post-synaptic terminals, and the resulting reduced dopaminergic tone, are thought to contribute to the depressive-like symptoms of cocaine withdrawal and relapse of cocaine abuse (Volkow et al., 1993; Thomas et al., 2001).

In substance use disorders, relapse can be triggered by drug-related cues that function as discriminative stimuli to signal contingencies of drug availability and promote drug-taking behavior. For example, following drug-associated cue presentation, the amygdala signals to dopaminergic cell bodies in the VTA (Nestler and Carlezon, 2006; Cleck and Blendy, 2008). These VTA dopaminergic neurons then signal to the NAcs to release DA, which triggers increased gamma-aminobutyric acid (GABA)-ergic input to the thalamus (Koob, 1992; Nestler and Carlezon, 2006). This GABAergic thalamic input leads to hypoactivation of the prefrontal cortex (PFC), impairing judgment and reasoning (Volkow and Morales, 2015). Thus, a combination of increased DA output in the mesolimbic pathway and decreased PFC activation in cortical pathways appear to result in increased drug-taking behavior. Curiously, various types of stressors have been shown to promote drug-taking behavior in preclinical models of drug relapse (Mantsch et al., 2016; Dong et al., 2017), further highlighting the interconnection between stress and reward pathways in the brain.

Mechanisms of Stress Response

The LHPA influences a variety of functions including the digestive system, immune system, reproductive system, mood and energy expenditure (Vázquez, 1998). The LHPA undergoes self-regulation through feedback and modulates the extrahypothalamic stress neurocircuit (Koob and Kreek, 2007). In addition, the LHPA activates the brain reward circuit (Koob and Kreek, 2007), bridging the interdependent relationship of glucocorticoids (GCs) and the dopaminergic system.

The LHPA is activated following hypothalamic release of corticotropin-releasing hormone (CRH) and vasopressin through a hypophyseal portal system to the anterior pituitary (Aguilera, 2011). CRH may be triggered by either internal or external cues. Synergistically interacting with vasopressin, CRH induces adrenocorticotrophic hormone (ACTH) release by the anterior pituitary. ACTH then acts on the adrenal gland inducing GC secretion into the bloodstream. Cortisol, the primary GC in humans, binds to the GC receptor (GR) in the brain and other end organ tissues facilitating the stress response. The LHPA modulates the stress response through negative feedback on the axis, specifically through negative feedback on the anterior pituitary and hypothalamus that inhibits ACTH and CRH release, ultimately decreasing blood cortisol levels through reduced release.

The GR is a transcription factor, and following translocation to the nucleus, the GR can modulate 10%–20% of genes in the human genome (Oakley and Cidlowski, 2013). While unbound GR remains in the cytosol, in the presence of cortisol, bound GR translocates to the nucleus and interacts with GC response elements (GREs) to modulate transcription (Chrousos et al., 2009). Moreover, GR interacts with other transcription factors, including nuclear factor-κB (NF-κB; Russo et al., 2007) and activator protein-1 (AP-1), which have been implicated in the progression to severe substance use disorder (Hope, 1998; Chrousos et al., 2009).

Interactions Between Glucocorticoids and the Dopaminergic System

The interactions between LHPA-induced GC release and the dopaminergic system are pivotal to understanding interactions between stress and substance use disorders. Both stressors and drugs of abuse have been shown to activate the mesolimbic "reward" pathway. For example, both increase glutamate receptor activation of VTA dopaminergic neurons (Cleck and Blendy, 2008). In addition, the LHPA axis also enhances glutamatergic plasticity in the VTA (Stelly et al., 2016). Furthermore, Barrot et al. (2000) have shown that adrenalectomy leading to decreased GC levels resulted in decreased basal and cocaine-induced increase in NAcs shell DA levels. **Figure 1** shows three potential mechanisms by which GCs are hypothesized to alter dopaminergic activity. First, GCs increase DA biosynthesis by enhancing tyrosine hydroxylase (TH) activity, the rate-limiting enzyme in DA synthesis (Daubner et al., 2011). This is illustrated by the observation that rats exposed to social isolation have increased TH levels in the NAcs shell (Trainor, 2011). A second mechanism by which GCs are hypothesized to alter dopaminergic activity is through GC-induced reductions in monoamine-oxidase (MAO) activity (Poletto et al., 2011). MAO is another method, in addition to monoamine reuptake by presynaptic transporters as described above, for terminating monoamine neurotransmission. Decreased MAO activity would increase synaptic DA levels and enhance dopaminergic neurotransmission. Lastly, GCs acting at GRs have been shown to regulate DA transporter expression under both basal and cocaine-stimulated conditions (Wheeler et al., 2017). These results are also consistent with reduced DA transporters in rats that underwent early life stress (Meaney et al., 2002). Overall, this literature supports a role of GC regulation of the mesolimbic DA pathway at multiple levels to alter both basal and cocaine-induced dopaminergic neurotransmission.

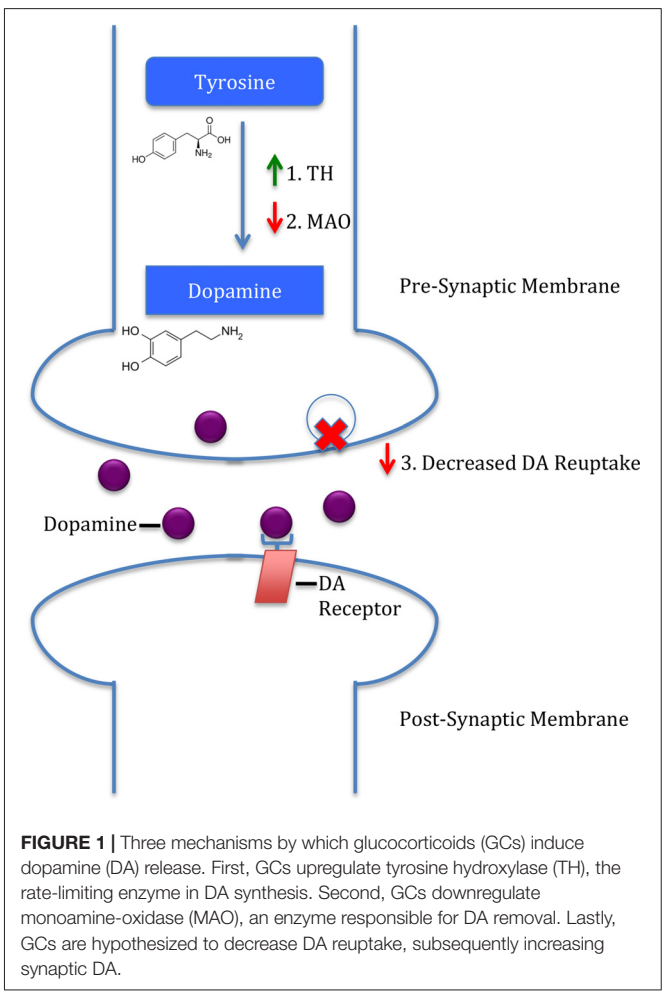

FIGURE 1 | Three mechanisms by which glucocorticoids (GCs) induce dopamine (DA) release. First, GCs upregulate tyrosine hydroxylase (TH), the rate-limiting enzyme in DA synthesis. Second, GCs downregulate monoamine-oxidase (MAO), an enzyme responsible for DA removal. Lastly, GCs are hypothesized to decrease DA reuptake, subsequently increasing synaptic DA.

VTA

Increased Glutamatergic Plasticity

Both stress and drugs of abuse have been shown to increase glutamatergic plasticity in the VTA (Saal et al., 2003). Furthermore, exposure to stressful events enhances VTA glutamatergic plasticity that may further enhance the abuse-related effects of cocaine (Fitzgerald et al., 1996; Kauer and Malenka, 2007; Stelly et al., 2016). In a recent study by Stelly et al. (2016), rats first underwent a resident-intruder social defeat paradigm in conjunction with corticosterone injections, and then cocaine rewarding effects were assessed using a conditioned place preference (CPP) procedure. Repeated social defeat selectively enhanced long-term potentiation (LTP) of N-Methyl-D-aspartic acid receptors (NMDARs) in the VTA. This LTP manifested as enhanced VTA dopaminergic neuron firing in response to cocaine-associated cues during CPP only in the stressed group. This additional dopaminergic burst was interpreted as enhancing the conditioned stimulus-response relationship between drug-associated cues and the abused drug that may be involved in drug relapse (Stelly et al., 2016). These results suggest stress-induced glutamatergic plasticity of NMDAR and subsequent enhancement of cocaine abuse-related effects may be attenuated in the VTA by a GC antagonist. Deletion of *nuclear receptor subfamily 3, group C, member 1 (nr3c1)*, a gene encoding a GR, blunted cocaine reinforcement in a drug self-administration procedure and VTA dopaminergic firing (Ambroggi et al., 2009; Barik et al., 2013). These results provide further evidence that GRs modulate VTA dopaminergic plasticity that directly impacts the abuse-related effects of cocaine.

Accumulating evidence suggests one molecular mechanism by which both stress and drugs of abuse impact glutamatergic plasticity in the mesolimbic pathway is through extracellular signal-regulated kinases (ERK). For example, stress exposure increased inositol 1,4,5-trisphosphate receptors (IP3R) sensitization that was mediated by protein kinase A (PKA), an upstream activator in ERK pathway (Vanhoutte et al., 1999; Stelly et al., 2016; **Figure 2**). Consistent with these previous results, social-defeat stress increased ERK signaling in the VTA (Yap et al., 2015). Moreover, ERK signaling appears to rely on the relative ratio of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and NMDARs.

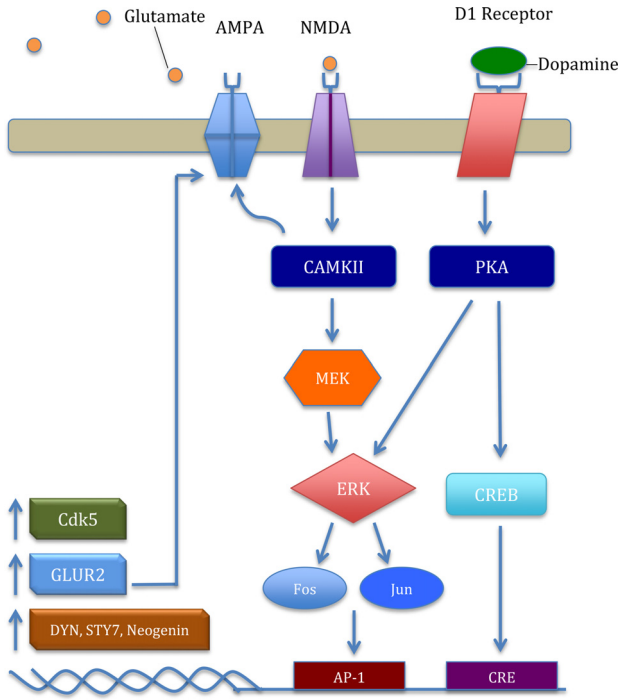

FIGURE 2 | The general mechanism of the extracellular signal-regulated kinase (ERK) pathway and key downstream products important in cocaine addiction. Glutamate binding excites the N-Methyl-D-aspartic acid receptor (NMDAR) and upregulates intracellular calcium. Excitation provokes a signaling cascade, upregulating transcription factors Fos and Jun. Subsequently, increased ΔFosB acts on activator protein-1 (AP-1) and upregulates transcription and translation of cyclin-dependent kinase 5 (CDK5), GLUR2, dynorphin (Dyn), synaptotagmin VII (Syt7), and neogenin. CDK5 mediates localization and GLUR2-mediated plasticity in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) through phosphorylation of δ-Catenin (Poore et al., 2010). Increased GLUR2 upregulates the ERK pathway in a positive-feedback type manner through increased AMPAR in the nucleus accumbens (NAcs). Moreover, increased D1 receptor activation upregulates protein kinase A (PKA), phosphorylating the transcription factor cAMP response element binding protein (CREB), leading to further increase in CDK5 and GLUR2 protein levels.

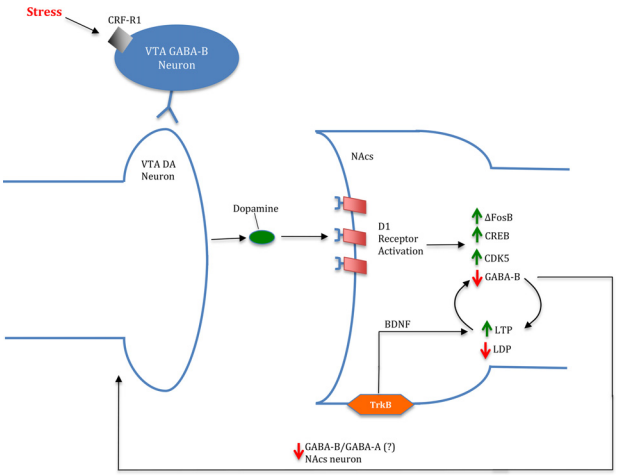

FIGURE 3 | Image of a potential mechanism for stress- and cocaine-induced drug dependence *via* a feed-forward cycle in the NAcs. In the presence of stress, ventral tegmental area (VTA) DA release is upregulated resulting in increased D1 receptor activation. Cortisol is implicated in increasing DA release through corticotropin-releasing factor acting at type 1 receptor (CRF-R1) binding to gamma-aminobutyric acid (GABA)-B VTA neurons acting on VTA DA neurons. Increased DA levels promote D1 activation leading to an increase in ΔFosB, CREB and CDK5 levels in the NAcs. Moreover, D1 activation is linked to decreased GABA-B activation in the NAcs, resulting in greater long-term potentiation (LTP): long-term depression (LDP). Attenuation of GABA projections from NAcs to the VTA is suggested to further DA release; however, the particular projection (GABA-A/GABA-B) is currently unknown. Furthermore, brain-derived neurotrophic factor (BDNF) is implicated in contributing to LTP in the NAcs through activation of tropomyosin receptor kinase B (TrkB) receptors.

For example, stress exposure increases the AMPA/NMDA ratio in the VTA (Saal et al., 2003; Dong et al., 2004). However, inhibition of ERK activation has produced equivocal results on the abuse-related effects of cocaine. Administration of SL327, a mitogen-activated protein kinase (MEK) inhibitor used to inhibit ERK, decreased both context and cocaine-induced CPP (Valjent et al., 2000, 2006; Pan et al., 2011). This trend may be indicative of neuroadaptive changes post ERK inhibition. In contrast, administration of U0126, another MEK inhibitor, directly into the VTA enhanced both context and cocaine cue-induced reinstatement in non-stressed rats (Lu et al., 2004, 2009). However, in rats undergoing social stress first, U0126 directly into the VTA attenuated stress-enhanced cocaine locomotor sensitization (Stelly et al., 2016). Taken together, the role of ERK activation in cocaine's abuse-related effects seems fundamental to understanding downstream physiological and behavioral alterations initiated in the VTA.

CRF-R1 Modulation

Corticotropin-releasing factor acting at type 1 receptor (CRF-R1) has also emerged as one potential molecular mechanism linking stress and drug abuse. For example, intermittent social defeat stress elicits CRF release in the VTA (Holly et al., 2016). Furthermore, social defeat stress or intra-VTA CRF enhanced the abuse-related effects of cocaine in rats (Boyson et al., 2014; Leonard et al., 2017). Consistent with these previous findings, administration of a CRF antagonist before each social defeat stress attenuated both cocaine-induced locomotor sensitization and escalated cocaine self-administration in rats (Boyson et al., 2011). However, CRF antagonists also decrease escalated cocaine self-administration in non-stressed rats (Specio et al., 2008) suggesting the role of CRF on interactions between social stress and cocaine abuse-related effects have not been fully elucidated. Further complicating the role of CRF in cocaine reinforcement are results from nonhuman primates demonstrating a CRF antagonist does not attenuate cocaine self-administration (Mello et al., 2006). In congruence with this observation, the CRF antagonist verucerfont failed to attenuate alcohol craving in anxious alcoholic women, despite blocking HPA axis responsivity to dexamethasone (Schwandt et al., 2016). Overall, in contrast to the preclinical reports using rodents, nonhuman primate and clinical results do not provide compelling evidence for a significant role of CRF in altering

the abuse-related effects of abused drugs in either stress or non-stressed research subjects.

NUCLEUS ACCUMBENS NAcs

Increased LTP From D1 Activation

In addition to drug-induced changes in the VTA, chronic cocaine use and stress exposure can directly alter the NAcs (Wolf and Ferrario, 2010; Koya and Hope, 2011). Preclinical models show cocaine-induced morphological changes in dendritic spine density and greater AMPAR/NMDAR firing in the NAcs after administration alone (Wolf and Ferrario, 2010; Koya and Hope, 2011). Furthermore, chronic stress may alter relapse and self-administration *via* epigenetic modifications to histone dimethyltransferase G9a in the NAcs (Anderson et al., 2018). In addition to drug and stress induced changes in the NAcs, chronic stress exposure may further substance abuse *via* a feedback loop with the VTA. The D1 receptor is a G_s-protein coupled post-synaptic receptor that is linked to upregulation of FBJ murine osteosarcoma viral oncogene homolog B (ΔFosB), cAMP response element binding protein (CREB), and cyclin-dependent kinase 5 (CDK5; Catalano et al., 2009; Lebel et al., 2009; Zhang et al., 2002). Increased D1 receptor activation leads to upregulated glutamatergic receptors in the NAcs (Chao et al., 2002; Mangiavacchi and Wolf, 2004). In addition, increased D1 activation attenuates GABA-B, a metabotropic transmembrane receptor, inhibition due to changes in adenosine levels after cocaine exposure in the VTA (Bonci and Williams, 1996). Reduced inhibition by GABA-B can subsequently increase LTP (Nicola et al., 2000) and decrease long-term depression (LDP) leading to increased synaptic plasticity in the NAcs (Bonci and Williams, 1996; Nicola et al., 2000; Fourgeaud et al., 2004). NAcs inhibitory neurons can project back to the VTA, resulting in a possible feedback loop of increased neurogenic excitability and DA release (Omelchenko and Sesack, 2009; Xia et al., 2011). The increase in potentiation further excites DA cells, causing DA release (Gonon and Sundstrom, 1996; Gonon, 1997). This theory aligns with recent data suggesting increased DA release after CGP55845 administration, a GABA-B antagonist (Melchior et al., 2015). Subsequently, greater DA in the synapse reduces D1 DA receptor availability in the ventral striatum according to recent PET scans (Martinez et al., 2009). Additional research is needed to support a pattern of a positive feedback loop and greater VTA response to the drug. Furthermore, stress-induced cocaine seeking is initiated by GABA-B receptor-dependent CRF actions in the VTA (Blacktop et al., 2016). Although this modulation by stress is carried out in the VTA, effects of GABA-B and CRF interactions are exerted in the postsynaptic membrane in the NAcs. Additional evidence suggests brain-derived neurotrophic factor (BDNF) may mediate neuronal excitability through activation of tropomyosin receptor kinase B (TrkB) receptors in the NAcs (Berton et al., 2006). Lobo et al. (2010) found a loss of TrkB receptors, mimicked through upregulation of D2 neurons, lead to decreased cocaine reward; in contrast, upregulation of D1 excitability showed an increase in cocaine reward. In addition to BDNF's mediating role, stress is implicated in facilitation of further synaptic adaptations in the NAcs. To this end, Chaudhury et al. (2013) demonstrated that repeated social defeat stress may induce VTA DA neuron phasic firing to the NAcs in mice. These data suggest that stress-induced phasic firing of the VTA may augment synaptic excitability in the NAcs of cocaine-addicted brains (Chaudhury et al., 2013).

The proposition that stress exerts effects through inhibition of positive feedback is not fully supported in the extant literature. For example, Sinha (2008), reported that chronic stress inhibits DA synthesis in the NAcs. However, it is well supported that GC concentrations directly correlate with extracellular DA release (Brake et al., 2004; Sinha, 2008). Although DA synthesis may be inhibited by chronic stress, cocaine sensitization has been repeatedly shown to increase by gene and protein regulators such as ΔFosB, CREB and CDK5 (Kelz et al., 1999; Bibb et al., 2001; McClung and Nestler, 2003; Mattson et al., 2005). Therefore, the combined data leads us to conclude that stress increases drug addiction susceptibility through increased sensitization in a positive feedback manner (**Figure 3**). Furthermore, the literature suggests that stress perpetuates drug dependence through allostasis by reinforcement in an analogous feedback manner (Koob and Le Moal, 2001; Ahmed et al., 2002). Taken together, the available findings collectively suggest that stress may mediate drug dependence at multiple levels, through positive feedback mechanisms.

CONCLUSION AND FUTURE DIRECTIONS

Although this mini-review article has focused on the effects of stress on the mesolimbic DA pathway, the effects of stress on other brain regions implicated in substance use disorders are important considerations beyond the capacity of this brief synopsis. For example, GRs are highly expressed in the PFC. GCs can act locally in the PFC to modulate cognitive impairments in working memory due to acute stress (Butts et al., 2011). Similar to GC effects on the mesolimbic DA pathway, corticosterone administered directly into the PFC can increase DA efflux (Butts et al., 2011). However, despite the relevant function of the PFC in substance use disorders (Volkow et al., 2016), relatively little research has been done to determine the extent to which molecular intermediaries such as ΔFosB, CREB, or CDK5 are involved in the PFC with regard to stress-induced enhancement of cocaine abuse-related effects.

Collectively, this mini-review article details three potential molecular mechanisms relating DA and GC interactions as they relate to stress-induced enhancement of cocaine abuse-related behaviors. In all three mechanisms, stress-induced GC release and subsequent activation of GRs primes the mesolimbic DA pathway. The overall net effect is enhanced abuse-related effects of cocaine and enhanced susceptibility of progressing to a cocaine use disorder diagnosis (Sinha, 2008). Thus, stress may serve as a positive feedback mechanism in the NAcs for enhancing the susceptibility to, or progression to, substance use disorder.

AUTHOR CONTRIBUTIONS

DM and GN discussed ideas for the initial submission. DM led the literature search and discussed results with GN. GN advised on direction and additional resources. DM wrote the majority of the initial manuscript and GN provided revisions and reformatting of content. MB provided substantial editorial comments following the first stage of review including writing additional content and recommendations on revisions for existing content. All individuals have approved the final version and agree to be responsible for the content.

FUNDING

This work was supported by the National Institutes of Health National Institute of Nursing Research (NR014886; GN).

REFERENCES

Aguilera, G. (2011). HPA axis responsiveness to stress: implications for healthy aging. *Exp. Gerontol.* 46, 90–95. doi: 10.1016/j.exger.2010.08.023

Ahmed, S. H., Kenny, P. J., Koob, G. F., and Markou, A. (2002). Neurobiological evidence for hedonic allostasis associated with escalating cocaine use. *Nat. Neurosci.* 5, 625–626. doi: 10.1038/nn872

Ambroggi, F., Turiault, M., Milet, A., Deroche-Gamonet, V., Parnaudeau, S., Balado, E., et al. (2009). Stress and addiction: glucocorticoid receptor in dopaminoceptive neurons facilitates cocaine seeking. *Nat. Neurosci.* 12, 247–249. doi: 10.1038/nn.2282

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders (DSM-5)®*. Arlington, VA: American Psychiatric Publishing.

Anderson, E. M., Larson, E. B., Guzman, D., Wissman, A. M., Neve, R. L., Nestler, E. J., et al. (2018). Overexpression of the histone dimethyltransferase G9a in nucleus accumbens shell increases cocaine self-administration, stress-induced reinstatement, and anxiety. *J. Neurosci.* 38, 803–813. doi: 10.1523/JNEUROSCI.1657-17.2017

Banks, M. L., Czoty, P. W., Gage, H. D., Bounds, M. C., Garg, P. K., Garg, S., et al. (2008). Effects of cocaine and MDMA self-administration on serotonin transporter availability in monkeys. *Neuropsychopharmacology* 33, 219–225. doi: 10.1038/sj.npp.1301420

Barik, J., Marti, F., Morel, C., Fernandez, S. P., Lanteri, C., Godeheu, G., et al. (2013). Chronic stress triggers social aversion via glucocorticoid receptor in dopaminoceptive neurons. *Science* 339, 332–335. doi: 10.1126/science.1226767

Barrot, M., Marinelli, M., Abrous, D. N., Rougé-Pont, F., Le Moal, M., and Piazza, P. V. (2000). The dopaminergic hyper-responsiveness of the shell of the nucleus accumbens is hormone-dependent. *Eur. J. Neurosci.* 12, 973–979. doi: 10.1046/j.1460-9568.2000.00996.x

Berton, O., McClung, C. A., DiLeone, R. J., Krishnan, V., Renthal, W., Russo, S. J., et al. (2006). Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science* 311, 864–868. doi: 10.1126/science.1120972

Beveridge, T. J., Smith, H. R., Nader, M. A., and Porrino, L. J. (2005). Effects of chronic cocaine self-administration on norepinephrine transporters in the nonhuman primate brain. *Psychopharmacology* 180, 781–788. doi: 10.1007/s00213-005-2162-1

Bibb, J. A., Chen, J., Taylor, J. R., Svenningsson, P., Nishi, A., Snyder, G. L., et al. (2001). Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. *Nature* 410, 376–380. doi: 10.1038/35066591

Blacktop, J. M., Vranjkovic, O., Mayer, M., Van Hoof, M., Baker, D. A., and Mantsch, J. R. (2016). Antagonism of GABA-B but not GABA-A receptors in the VTA prevents stress- and intra-VTA CRF-induced reinstatement of extinguished cocaine seeking in rats. *Neuropharmacology* 102, 197–206. doi: 10.1016/j.neuropharm.2015.11.013

Bonci, A., and Williams, J. T. (1996). A common mechanism mediates long-term changes in synaptic transmission after chronic cocaine and morphine. *Neuron* 16, 631–639. doi: 10.1016/s0896-6273(00)80082-3

Boyson, C. O., Holly, E. N., Shimamoto, A., Albrechet-Souza, L., Weiner, L. A., DeBold, J. F., et al. (2014). Social stress and CRF–dopamine interactions in the VTA: role in long-term escalation of cocaine self-administration. *J. Neurosci.* 34, 6659–6667. doi: 10.1523/JNEUROSCI.3942-13.2014

Boyson, C. O., Miguel, T. T., Quadros, I. M., Debold, J. F., and Miczek, K. A. (2011). Prevention of social stress-escalated cocaine self-administration by CRF-R1 antagonist in the rat VTA. *Psychopharmacology* 218, 257–269. doi: 10.1007/s00213-011-2266-8

Brake, W. G., Zhang, T. Y., Diorio, J., Meaney, M. J., and Gratton, A. (2004). Influence of early postnatal rearing conditions on mesocorticolimbic dopamine and behavioural responses to psychostimulants and stressors in adult rats. *Eur. J. Neurosci.* 19, 1863–1874. doi: 10.1111/j.1460-9568.2004.03286.x

Butts, K. A., Weinberg, J., Young, A. H., and Phillips, A. G. (2011). Glucocorticoid receptors in the prefrontal cortex regulate stress-evoked dopamine efflux and aspects of executive function. *Proc. Natl. Acad. Sci. U S A* 108, 18459–18464. doi: 10.1073/pnas.1111746108

Catalano, S., Giordano, C., Rizza, P., Gu, G., Barone, I., Bonofiglio, D., et al. (2009). Evidence that leptin through STAT and CREB signaling enhances cyclin D1 expression and promotes human endometrial cancer proliferation. *J. Cell. Physiol.* 218, 490–500. doi: 10.1002/jcp.21622

Chao, S. Z., Ariano, M. A., Peterson, D. A., and Wolf, M. E. (2002). D1 dopamine receptor stimulation increases GluR1 surface expression in nucleus accumbens neurons. *J. Neurochem.* 83, 704–712. doi: 10.1046/j.1471-4159.2002.01164.x

Chaudhury, D., Walsh, J. J., Friedman, A. K., Juarez, B., Ku, S. M., Koo, J. W., et al. (2013). Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. *Nature* 493, 532–536. doi: 10.1038/nature11713

Chrousos, G. P., Kino, T., and Charmandari, E. (2009). Evaluation of the hypothalamic-pituitary-adrenal axis function in childhood and adolescence. *Neuroimmunomodulation* 16, 272–283. doi: 10.1159/000216185

Cleck, J. N., and Blendy, J. A. (2008). Making a bad thing worse: adverse effects of stress on drug addiction. *J. Clin. Invest.* 118, 454–461. doi: 10.1172/jci33946

Czoty, P. W., Gage, H. D., Nader, S. H., Reboussin, B. A., Bounds, M., and Nader, M. A. (2007). PET imaging of dopamine D2 receptor and transporter availability during acquisition of cocaine self-administration in rhesus monkeys. *J. Addict. Med.* 1, 33–39. doi: 10.1097/adm.0b013e318045c038

Daubner, S. C., Le, T., and Wang, S. (2011). Tyrosine hydroxylase and regulation of dopamine synthesis. *Arch. Biochem. Biophys.* 508, 1–12. doi: 10.1016/j.abb.2010.12.017

Dong, Y., Saal, D., Thomas, M., Faust, R., Bonci, A., Robinson, T., et al. (2004). Cocaine-induced potentiation of synaptic strength in dopamine neurons: behavioral correlates in GluRA (−/−) mice. *Proc. Natl. Acad. Sci. U S A* 101, 14282–14287. doi: 10.1073/pnas.0401553101

Dong, Y., Taylor, J. R., Wolf, M. E., and Shaham, Y. (2017). Circuit and synaptic plasticity mechanisms of drug relapse. *J. Neurosci.* 37, 10867–10876. doi: 10.1523/JNEUROSCI.1821-17.2017

Ewing Corcoran, S. B., and Howell, L. L. (2010). Impact of early life stress on the reinforcing and behavioral-stimulant effects of psychostimulants in rhesus monkeys. *Behav. Pharmacol.* 21, 69–76. doi: 10.1097/fbp.0b013e3283359f53

Fitzgerald, L. W., Ortiz, J., Hamedani, A. G., and Nestler, E. J. (1996). Drugs of abuse and stress increase the expression of GluR1 and NMDAR1 glutamate receptor subunits in the rat ventral tegmental area: common adaptations among cross-sensitizing agents. *J. Neurosci.* 16, 274–282. doi: 10.1523/JNEUROSCI.16-01-00274.1996

Fourgeaud, L., Mato, S., Bouchet, D., Hémar, A., Worley, P. F., and Manzoni, O. J. (2004). A single *in vivo* exposure to cocaine abolishes endocannabinoid-mediated long-term depression in the nucleus accumbens. *J. Neurosci.* 24, 6939–6945. doi: 10.1523/JNEUROSCI.0671-04.2004

Gonon, F. (1997). Prolonged and extrasynaptic excitatory action of dopamine mediated by D1 receptors in the rat striatum *in vivo*. *J. Neurosci.* 17, 5972–5978. doi: 10.1523/JNEUROSCI.17-15-05972.1997

Gonon, F., and Sundstrom, L. (1996). Excitatory effects of dopamine released by impulse flow in the rat nucleus accumbens *in vivo*. *Neuroscience* 75, 13–18. doi: 10.1016/0306-4522(96)00320-x

Holly, E. N., Boyson, C. O., Montagud-Romero, S., Stein, D. J., Gobrogge, K. L., DeBold, J. F., et al. (2016). Episodic social stress-escalated cocaine self-administration: role of phasic and tonic corticotropin releasing factor in the

anterior and posterior ventral tegmental area. *J. Neurosci.* 36, 4093–4105. doi: 10.1523/JNEUROSCI.2232-15.2016

Hope, B. T. (1998). Cocaine and the AP-1 transcription factor complex. *Ann. N Y Acad. Sci.* 844, 1–6. doi: 10.1111/j.1749-6632.1998.tb08216.x

Kauer, J. A., and Malenka, R. C. (2007). Synaptic plasticity and addiction. *Nat. Rev. Neurosci.* 8, 844–858. doi: 10.1038/nrn2234

Kelz, M. B., Chen, J., Carlezon, W. A. Jr., Whisler, K., Gilden, L., Beckmann, A. M., et al. (1999). Expression of the transcription factor ΔFosB in the brain controls sensitivity to cocaine. *Nature* 401, 272–276. doi: 10.1038/45790

Koob, G. F. (1992). Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends Pharmacol. Sci.* 13, 177–184. doi: 10.1016/0165-6147(92)90060-j

Koob, G. F. (2009). Dynamics of neuronal circuits in addiction: reward, antireward, and emotional memory. *Pharmacopsychiatry* 42, S32–S41. doi: 10.1055/s-0029-1216356

Koob, G. F. (2011). Theoretical frameworks and mechanistic aspects of alcohol addiction: alcohol addiction as a reward deficit disorder. *Curr. Top. Behav. Neurosci.* 13, 3–30. doi: 10.1007/7854_2011_129

Koob, G. F., and Le Moal, M. (2001). Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology* 24, 97–129. doi: 10.1016/s0893-133x(00)00195-0

Koob, G., and Kreek, M. J. (2007). Stress, dysregulation of drug reward pathways, and the transition to drug dependence. *Am. J. Psychiatry* 164, 1149–1159. doi: 10.1176/appi.ajp.2007.05030503

Kosten, T. A., Miserendino, M. J., and Kehoe, P. (2000). Enhanced acquisition of cocaine self-administration in adult rats with neonatal isolation stress experience. *Brain Res.* 875, 44–50. doi: 10.1016/s0006-8993(00)02595-6

Koya, E., and Hope, B. T. (2011). Cocaine and synaptic alterations in the nucleus accumbens. *Biol. Psychiatry* 69, 1013–1014. doi: 10.1016/j.biopsych.2011.04.001

Laurier, L. G., Corrigall, W. A., and George, S. R. (1994). Dopamine receptor density, sensitivity and mRNA levels are altered following self-administration of cocaine in the rat. *Brain Res.* 634, 31–40. doi: 10.1016/0006-8993(94)90255-0

Lebel, M., Patenaude, C., Allyson, J., Massicotte, G., and Cyr, M. (2009). Dopamine D1 receptor activation induces tau phosphorylation via cdk5 and GSK3 signaling pathways. *Neuropharmacology* 57, 392–402. doi: 10.1016/j.neuropharm.2009.06.041

Leonard, M. Z., DeBold, J. F., and Miczek, K. A. (2017). Escalated cocaine "binges" in rats: enduring effects of social defeat stress or intra-VTA CRF. *Psychopharmacology* 234, 2823–2836. doi: 10.1007/s00213-017-4677-7

Lobo, M. K., Covington, H. E. III., Chaudhury, D., Friedman, A. K., Sun, H., Damez-Werno, D., et al. (2010). Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. *Science* 330, 385–390. doi: 10.1126/science.1188472

Lu, L., Dempsey, J., Liu, S. Y., Bossert, J. M., and Shaham, Y. (2004). A single infusion of brain-derived neurotrophic factor into the ventral tegmental area induces long-lasting potentiation of cocaine seeking after withdrawal. *J. Neurosci.* 24, 1604–1611. doi: 10.1523/JNEUROSCI.5124-03.2004

Lu, L., Wang, X., Wu, P., Xu, C., Zhao, M., Morales, M., et al. (2009). Role of ventral tegmental area glial cell line-derived neurotrophic factor in incubation of cocaine craving. *Biol. Psychiatry* 66, 137–145. doi: 10.1016/j.biopsych.2009.02.009

Macey, D. J., Smith, H. R., Nader, M. A., and Porrino, L. J. (2003). Chronic cocaine self-administration upregulates the norepinephrine transporter and alters functional activity in the bed nucleus of the stria terminalis of the rhesus monkey. *J. Neurosci.* 23, 12–16. doi: 10.1523/JNEUROSCI.23-01-00012.2003

Mangiavacchi, S., and Wolf, M. E. (2004). D1 dopamine receptor stimulation increases the rate of AMPA receptor insertion onto the surface of cultured nucleus accumbens neurons through a pathway dependent on protein kinase A. *J. Neurochem.* 88, 1261–1271. doi: 10.1046/j.1471-4159.2003.02248.x

Mantsch, J. R., Baker, D. A., Funk, D., Lê, A. D., and Shaham, Y. (2016). Stress-induced reinstatement of drug seeking: 20 years of progress. *Neuropsychopharmacology* 41, 335–356. doi: 10.1038/npp.2015.142

Martinez, D., Slifstein, M., Narendran, R., Foltin, R. W., Broft, A., Hwang, D. R., et al. (2009). Dopamine D1 receptors in cocaine dependence measured with PET and the choice to self-administer cocaine. *Neuropsychopharmacology* 34, 1774–1782. doi: 10.1038/npp.2008.235

Mattson, B. J., Bossert, J. M., Simmons, D. E., Nozaki, N., Nagarkar, D., Kreuter, J. D., et al. (2005). Cocaine-induced CREB phosphorylation in nucleus accumbens of cocaine-sensitized rats is enabled by enhanced activation of extracellular signal-related kinase, but not protein kinase A. *J. Neurochem.* 95, 1481–1494. doi: 10.1111/j.1471-4159.2005.03500.x

McClung, C. A., and Nestler, E. J. (2003). Regulation of gene expression and cocaine reward by CREB and ΔFosB. *Nat. Neurosci.* 6, 1208–1215. doi: 10.1038/nn1143

Meaney, M. J., Brake, W., and Gratton, A. (2002). Environmental regulation of the development of mesolimbic dopamine systems: a neurobiological mechanism for vulnerability to drug abuse? *Psychoneuroendocrinology* 27, 127–138. doi: 10.1016/s0306-4530(01)00040-3

Melchior, J. R., Ferris, M. J., Stuber, G. D., Riddle, D. R., and Jones, S. R. (2015). Optogenetic versus electrical stimulation of dopamine terminals in the nucleus accumbens reveals local modulation of presynaptic release. *J. Neurochem.* 134, 833–844. doi: 10.1111/jnc.13177

Mello, N. K., Negus, S. S., Rice, K. C., and Mendelson, J. H. (2006). Effects of the CRF1 antagonist antalarmin on cocaine self-administration and discrimination in rhesus monkeys. *Pharmacol. Biochem. Behav.* 85, 744–751. doi: 10.1016/j.pbb.2006.11.008

Morgan, D., Grant, K. A., Gage, H. D., Mach, R. H., Kaplan, J. R., Prioleau, O., et al. (2002). Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. *Nat. Neurosci.* 5, 169–174. doi: 10.1038/nn798

Nader, M. A., Morgan, D., Gage, H. D., Nader, S. H., Calhoun, T. L., Buchheimer, N., et al. (2006). PET imaging of dopamine D2 receptors during chronic cocaine self-administration in monkeys. *Nat. Neurosci.* 9, 1050–1056. doi: 10.1038/nn1737

Nestler, E. J., and Carlezon, W. A. (2006). The mesolimbic dopamine reward circuit in depression. *Biol. Psychiatry* 59, 1151–1159. doi: 10.1016/j.biopsych.2005.09.018

Nicola, S. M., Surmeier, D. J., and Malenka, R. C. (2000). Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Annu. Rev. Neurosci.* 23, 185–215. doi: 10.1146/annurev.neuro.23.1.185

Oakley, R. H., and Cidlowski, J. A. (2013). The biology of the glucocorticoid receptor: new signaling mechanisms in health and disease. *J. Allergy Clin. Immunol.* 132, 1033–1044. doi: 10.1016/j.jaci.2013.09.007

Omelchenko, N., and Sesack, S. R. (2009). Ultrastructural analysis of local collaterals of rat ventral tegmental area neurons: GABA phenotype and synapses onto dopamine and GABA cells. *Synapse* 63, 895–906. doi: 10.1002/syn.20668

Pan, B., Zhong, P., Sun, D., and Liu, Q. S. (2011). Extracellular signal-regulated kinase signaling in the ventral tegmental area mediates cocaine-induced synaptic plasticity and rewarding effects. *J. Neurosci.* 31, 11244–11255. doi: 10.1523/JNEUROSCI.1040-11.2011

Piazza, P. V., and Le Moal, M. (1998). The role of stress in drug self-administration. *Trends Pharmacol. Sci.* 19, 67–74. doi: 10.1016/s0165-6147(97)01115-2

Poletto, R., Cheng, H. W., Meisel, R. L., Richert, B. T., and Marchant-Forde, J. N. (2011). Gene expression of serotonin and dopamine receptors and monoamine oxidase-A in the brain of dominant and subordinate pubertal domestic pigs (Sus scrofa) fed a β-adrenoreceptor agonist. *Brain Res.* 1381, 11–20. doi: 10.1016/j.brainres.2010.11.035

Poore, C. P., Sundaram, J. R., Pareek, T. K., Fu, A., Amin, N., Mohamed, N. E., et al. (2010). Cdk5-mediated phosphorylation of δ-catenin regulates its localization and GluR2-mediated synaptic activity. *J. Neurosci.* 30, 8457–8467. doi: 10.1523/JNEUROSCI.6062-09.2010

Quintero, G. C. (2013). Role of nucleus accumbens glutamatergic plasticity in drug addiction. *Neuropsychiatr. Dis. Treat.* 9, 1499–1512. doi: 10.2147/ndt.s45963

Ritz, M. C., Lamb, R. J., Goldberg, S. R., and Kuhar, M. J. (1987). Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* 237, 1219–1223. doi: 10.1126/science.2820058

Russo, S. J., Rental, W., and Kumar, A. (2007). *NFkB Signaling Regulates Cocaine-Induced Behavioral and Cellular Plasticity.* San Diego, CA: Society for Neuroscience.

Saal, D., Dong, Y., Bonci, A., and Malenka, R. C. (2003). Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. *Neuron* 37, 577–582. doi: 10.1016/s0896-6273(03)00021-7

Schwandt, M. L., Cortes, C. R., Kwako, L. E., George, D. T., Momenan, R., Sinha, R., et al. (2016). The CRF1 antagonist verucerfont in anxious alcohol -dependent women: translation of neuroendocrine, but not of anti-craving effects. *Neuropsychopharmacology* 41, 2818–2829. doi: 10.1038/npp.2016.61

Shield, K. D., Imtiaz, S., Probst, C., and Rehm, J. (2018). "The epidemiology and public health burden of addictive disorders," in *Integrating Psychological and Pharmacological Treatments for Addictive Disorders: An Evidence-Based Guide*, eds J. MacKillop, G. A. Kenna, L. Leggio and L. A. Ray (New York, NY: Routledge/Taylor & Francis Group), 3–31.

Sinha, R. (2001). How does stress increase risk of drug abuse and relapse? *Psychopharmacology* 158, 343–359. doi: 10.1007/s002130100917

Sinha, R. (2008). Chronic stress, drug use and vulnerability to addiction. *Ann. N Y Acad. Sci.* 1141, 105–130. doi: 10.1196/annals.1441.030

Specio, S. E., Wee, S., O'Dell, L. E., Boutrel, B., Zorrilla, E. P., and Koob, G. F. (2008). CRF_1 receptor antagonists attenuate escalated cocaine self-administration in rats. *Psychopharmacology* 196, 473–482. doi: 10.1007/s00213-007-0983-9

Stelly, C. E., Pomrenze, M. B., Cook, J. B., and Morikawa, H. (2016). Repeated social defeat stress enhances glutamatergic synaptic plasticity in the VTA and cocaine place conditioning. *Elife* 5:e15448. doi: 10.7554/elife.15448

Thomas, M. J., Beurrier, C., Bonci, A., and Malenka, R. C. (2001). Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. *Nat. Neurosci.* 4, 1217–1223. doi: 10.1038/nn757

Trainor, B. C. (2011). Stress responses and the mesolimbic dopamine system: social contexts and sex differences. *Horm. Behav.* 60, 457–469. doi: 10.1016/j.yhbeh.2011.08.013

Valjent, E., Corbillé, A. G., Bertran-Gonzalez, J., Hervé, D., and Girault, J. A. (2006). Inhibition of ERK pathway or protein synthesis during reexposure to drugs of abuse erases previously learned place preference. *Proc. Natl. Acad. Sci. U S A* 103, 2932–2937. doi: 10.1073/pnas.0511030103

Valjent, E., Corvol, J. C., Pagès, C., Besson, M. J., Maldonado, R., and Caboche, J. (2000). Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. *J. Neurosci.* 20, 8701–8709. doi: 10.1523/jneurosci.20-23-08701.2000

Vanhoutte, P., Barnier, J. V., Guibert, B., Pagès, C., Besson, M. J., Hipskind, R. A., et al. (1999). Glutamate induces phosphorylation of Elk-1 and CREB, along with c-fos activation, via an extracellular signal-regulated kinase-dependent pathway in brain slices. *Mol. Cell. Biol.* 19, 136–146. doi: 10.1128/mcb.19.1.136

Vázquez, D. M. (1998). Stress and the developing limbic-hypothalamic-pituitary-adrenal axis. *Psychoneuroendocrinology* 23, 663–700. doi: 10.1016/s0306-4530(98)00029-8

Volkow, N. D., Fowler, J. S., Wang, G. J., Hitzemann, R., Logan, J., Schlyer, D. J., et al. (1993). Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. *Synapse* 14, 169–177. doi: 10.1002/syn.890140210

Volkow, N. D., Fowler, J. S., Wolf, A. P., Schlyer, D., and Shiue, C. Y. (1990). Effects of chronic cocaine abuse on postsynaptic dopamine receptors. *Am. J. Psychiatry* 147, 719–724;. doi: 10.1176/ajp.147.6.719

Volkow, N. D., Koob, G. F., and McLellan, A. T. (2016). Neurobiologic advances from the brain disease model of addiction. *N. Engl. J. Med.* 374, 363–371. doi: 10.1056/nejmra1511480

Volkow, N., and Morales, M. (2015). The brain on drugs: from reward to addiction. *Cell* 162, 712–725. doi: 10.1016/j.cell.2015.07.046

Volkow, N. D., Wang, G. J., Fischman, M. W., Foltin, R. W., Fowler, J. S., Abumrad, N. N., et al. (1997). Relationship between subjective effects of cocaine and dopamine transporter occupancy. *Nature* 386, 827–830. doi: 10.1038/386827a0

Wang, G. J., Volkow, N. D., Fowler, J. S., Fischman, M., Foltin, R., Abumrad, N. N., et al. (1997). Cocaine abusers do not show loss of dopamine transporters with age. *Life Sci.* 61, 1059–1065. doi: 10.1016/s0024-3205(97)00614-0

Wheeler, D. S., Ebben, A. L., Kurtoglu, B., Lovell, M. E., Bohn, A. T., Jasek, I. A., et al. (2017). Corticosterone regulates both naturally occurring and cocaine-induced dopamine signaling by selectively decreasing dopamine uptake. *Eur. J. Neurosci.* 46, 2638–2646. doi: 10.1111/ejn.13730

Wolf, M. E., and Ferrario, C. R. (2010). AMPA receptor plasticity in the nucleus accumbens after repeated exposure to cocaine. *Neurosci. Biobehav. Rev.* 35, 185–211. doi: 10.1016/j.neubiorev.2010.01.013

Xia, Y., Driscoll, J. R., Wilbrecht, L., Margolis, E. B., Fields, H. L., and Hjelmstad, G. O. (2011). Nucleus accumbens medium spiny neurons target non-dopaminergic neurons in the ventral tegmental area. *J. Neurosci.* 31, 7811–7816. doi: 10.1523/jneurosci.1504-11.2011

Yap, J. J., Chartoff, E. H., Holly, E. N., Potter, D. N., Carlezon, W. A., and Miczek, K. A. (2015). Social defeat stress-induced sensitization and escalated cocaine self-administration: the role of ERK signaling in the rat ventral tegmental area. *Psychopharmacology* 232, 1555–1569. doi: 10.1007/s00213-014-3796-7

Zhang, D., Zhang, L., Lou, D. W., Nakabeppu, Y., Zhang, J., and Xu, M. (2002). The dopamine D1 receptor is a critical mediator for cocaine-induced gene expression. *J. Neurochem.* 82, 1453–1464. doi: 10.1046/j.1471-4159.2002.01089.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Behavioral Neuroscience

REVIEW
published: 26 October 2018
doi: 10.3389/fnbeh.2018.00240

Putative Inflammatory Sensitive Mechanisms Underlying Risk or Resilience to Social Stress

Julie E. Finnell[1] *and Susan K. Wood*[1,2]*

[1]Department of Pharmacology, Physiology, and Neuroscience, University of South Carolina School of Medicine, Columbia, SC, United States, [2]WJB Dorn Veterans Administration Medical Center, Columbia, SC, United States

It has been well recognized that exposure to stress can lead to the onset of psychosocial disorders such as depression. While there are a number of antidepressant therapies currently available and despite producing immediate neurochemical alterations, they require weeks of continuous use in order to exhibit antidepressant efficacy. Moreover, up to 30% of patients do not respond to typical antidepressants, suggesting that our understanding of the pathophysiology underlying stress-induced depression is still limited. In recent years inflammation has become a major focus in the study of depression as several clinical and preclinical studies have demonstrated that peripheral and central inflammatory mediators, including interleukin (IL)-1β, are elevated in depressed patients. Moreover, it has been suggested that inflammation and particularly neuroinflammation may be a direct and immediate link in the emergence of stress-induced depression due to the broad neural and glial effects that are elicited by proinflammatory cytokines. Importantly, individual differences in inflammatory reactivity may further explain why certain individuals exhibit differing susceptibility to the consequences of stress. In this review article, we discuss sources of individual differences such as age, sex and coping mechanisms that are likely sources of distinct changes in stress-induced neuroimmune factors and highlight putative sources of exaggerated neuroinflammation in susceptible individuals. Furthermore, we review the current literature of specific neural and glial mechanisms that are regulated by stress and inflammation including mitochondrial function, oxidative stress and mechanisms of glutamate excitotoxicity. Taken together, the impetus for this review is to move towards a better understanding of mechanisms regulated by inflammatory cytokines and chemokines that are capable of contributing to the emergence of depressive-like behaviors in susceptible individuals.

Keywords: stress susceptibility, neuroinflammation, depression, microglia, glutamate

OPEN ACCESS

Edited by:
Gretchen N. Neigh,
Virginia Commonwealth University,
United States

Reviewed by:
Leah M. Pyter,
The Ohio State University,
United States
Caroline Menard,
Université Laval, Canada

***Correspondence:**
Susan K. Wood
susan.wood@uscmed.sc.edu

Received: *28 June 2018*
Accepted: *25 September 2018*
Published: *26 October 2018*

Citation:
Finnell JE and Wood SK (2018) Putative Inflammatory Sensitive Mechanisms Underlying Risk or Resilience to Social Stress. Front. Behav. Neurosci. 12:240. doi: 10.3389/fnbeh.2018.00240

INTRODUCTION

Depression is considered to be one of the most debilitating diseases in the United States (Almeida, 2005) and has been globally recognized as a significant source of disability (Reddy, 2010). The prevalence of depression has been steadily increasing over the last 10 years from 6.6% to 7.3% in adults and 8.7%–12.7% in adolescents (Weinberger et al., 2017). While there are a number of available antidepressant therapies, many like the selective serotonin re-uptake inhibitor citalopram, are only 33% effective in producing full remission of depressive symptoms (Trivedi et al., 2006). Moreover, up to 30% of depressed patients are resistant to traditional

antidepressant therapies (Joffe et al., 1996; Al-Harbi, 2012). These data strongly suggest that the pathophysiology underlying the emergence of depression is variable between individuals and is still largely unclear. It was first noted that activation of the immune system impacted psychiatric functioning back in 1927 when Julius Wagner-Jauregg won the Nobel Prize for this seminal observation. Since this initial discovery, there has been a striking increase in the number of publications on the topic of inflammation related depression (Loftis et al., 2010). These studies have demonstrated that certain subpopulations of depressed patients exhibit greater levels of interleukin (IL)-6 and C reactive protein (CRP) in the plasma (Irwin and Miller, 2007) and cerebrospinal fluid (Sasayama et al., 2013; Devorak et al., 2015). Importantly, this vast body of literature has also established a causal link between inflammation and depression. Several clinical studies demonstrated that chronic administration of the cytokines interferon (INF)-α and IL-2 as chemotherapeutics were capable of inducing depression in a large number of patients (Denicoff et al., 1987; Renault et al., 1987). Moreover, it should be noted that individuals with inflammatory diseases such as irritable bowel disease, allergic rhinitis and rheumatoid arthritis (Cuffel et al., 1999; Stauder and Kovács, 2003; Katon et al., 2004; Marrie et al., 2017) as well as cardiovascular disease (Anda et al., 1993; Riba et al., 2011; Huffman et al., 2013) are at increased risk of developing psychiatric comorbidities.

Beyond immune diseases as a risk factor for psychiatric disorders, it has been well established that exposure to stress can also serve as an independent risk factor for the emergence of psychosocial disorders. While there are many different types of stress, social stressors such as bullying, abuse, isolation, witnessing traumatic events, or taking care of a terminally ill loved one are the most common types of stress encountered by people (Almeida, 2005). Importantly, it has been shown that exposure to social stress can not only produce increases in markers of inflammatory activity (Slavich et al., 2010; Allen et al., 2017) but can also augment underlying inflammatory disorders including allergic responses (Sandberg et al., 2000; Liu et al., 2002; Kiecolt-Glaser et al., 2008). However, preclinical and clinical studies have shown that there is considerable individual variability in the behavioral and inflammatory consequences induced by stress exposure resulting in the emergence of resilient and susceptible subpopulations. Specifically, it has been shown that greater inflammatory responses to stress are associated with greater negative affect in humans (Dickerson et al., 2009) and promote the development of depressive-like behaviors in rodents (Wohleb et al., 2013, 2014a,b; Hodes et al., 2014; Wood et al., 2015; Finnell and Wood, 2016; Finnell et al., 2017a,b, 2018). These stress-induced inflammatory effects are known to extend well beyond the immediate response to stress such that late phase inflammatory responses are also enhanced following social stress exposure (Kiecolt-Glaser et al., 2008; Deak et al., 2017). These late phase inflammatory effects have been tied to the emergence of chronic elevations of inflammatory factors through the recruitment and sensitization of inflammatory competent cell types including peripherally derived T cells (Janeway et al., 2001; Hansen et al., 2004) and microglia (Badoer, 2010).

Activation or sensitization of microglia, the resident immune cells of the brain, is of particular relevance to depression as a recent clinical study showed for the first time that depressed patients exhibit significant increases in translocator protein density, a marker of activated microglia (Setiawan et al., 2015). Under normal resting conditions, microglia exhibit a highly ramified morphology that is associated with monitoring and maintenance of the neural cell microenvironment (Nimmerjahn et al., 2005; Kettenmann et al., 2011). In response to a stress or immune challenge, these cell types take on an ameboid morphology that is associated with a reactive inflammatory state (Gemma and Bachstetter, 2013; Brites and Fernandes, 2015) resulting in the release of a number of different effectors including cytokines and chemokines (Brites and Fernandes, 2015). In this way reactive microglia are known to propagate inflammatory signals throughout the brain (Fruhbeis et al., 2013). However, the discrete neural mechanisms that may be impacted by the release of cytokines and chemokines in susceptible individuals remains unclear. Therefore, the focus of this review is to first provide an overview of the sources of individual differences in stress and inflammatory responses and second, to highlight discrete neural and glial mechanisms that are regulated by inflammatory effectors that may contribute to the emergence of behavioral dysfunction associated with a depressive-like state. Great focus has been placed on clinical and preclinical studies documenting the effects of social stress. However, other modalities of stress are discussed in instances where literature using social stress models is lacking.

SOURCES OF INDIVIDUAL DIFFERENCES IN INFLAMMATORY STRESS RESPONSES

Prior to beginning a discussion on the discrete neural mechanisms that may underlie the emergence of inflammatory related depressive-like behavior, it is critical to understand how individual factors such as age, sex and inherent differences in personality or coping may differentially impact the inflammatory system thereby contributing to stress susceptibility or resiliency.

Age

Stress susceptibility is well known to change across the lifespan. Importantly, life stages in which the brain is undergoing significant alterations, such as neural development and maturation in the young and senescence in the elderly (Graham et al., 2006), are associated with heightened susceptibility to the consequences of stress exposure. Much like stress susceptibility, immune function is also known to change across the lifespan. In general, innate and adaptive immune function decreases as individuals age (Lord et al., 2001; Gomez et al., 2005), resulting in dysregulated inflammatory responses to stress or immune challenges (Lord et al., 2001). For example, studies in rodents have indicated that aged rats do not develop inflammatory tolerance to repeated lipopolysaccharide (LPS) injections as is observed in younger rats (Li et al., 2009). Moreover, LPS

inflammatory reactivity has also been shown to be greater in middle-aged mice compared with young mice (Kohman et al., 2010). This increase in inflammatory reactivity in aged animals has also been demonstrated in the brain as a result of natural microglial shifts towards a "primed" phenotype (Barrientos et al., 2015). Heightened inflammatory sensitivity in aging populations, termed inflammatory senescence, also extends to the inflammatory response to stress. Specifically, it has been shown that transient stressors more commonly produce maladaptive inflammatory responses in the elderly compared to younger individuals (Segerstrom and Miller, 2004). Moreover, exposure to stress can also accelerate the process of inflammatory senescence. This assumption is supported by a prospective clinical study which determined that older adults serving as care givers exhibited a four-fold faster elevation in resting plasma IL-6 over a 6-year period compared to age-matched non-caregivers (Kiecolt-Glaser et al., 2003). While clinical studies assessing stress responsivity in aging populations are relatively limited, it is well recognized that social stress and particularly social isolation is extremely common especially for those living in retirement communities. This is of particular importance as approximately 15% of elderly individuals living in retirement communities exhibit significant depressive symptomatology and are more likely to exhibit suicidal tendencies (Fiske et al., 2009). Based on the strong role that stress-induced inflammation is suggested to play in the emergence of depressive-like behavioral states, it is possible that inflammatory senescence may represent a putative mechanism underlying the emergence of depression in aged populations.

Younger populations on the other hand generally exhibit greater resilience to immune challenges while simultaneously exhibiting enhanced behavioral susceptibility to stress. At a cursory glance these effects seem to be opposing. However, these data do not consider the detrimental effects that inflammation produces in the developing organism. Specifically, it has been shown that stress (Bath et al., 2016) and inflammation (Johnson and Kaffman, 2018) at early developmental stages can significantly alter the function, maturation and proliferation of neurons and glia. Moreover, exposure to early life stress is known to promote shifts in the function of immune cells that are resistant to alterations later in life (Lubach et al., 1995), suggesting that early life stress results in long-term reprogramming of the immune system. This assumption has been verified by several studies demonstrating that early life stress not only increases the susceptibility to developing autoimmune deficiencies (Capitanio and Lerche, 1991) but also produces sensitization to subsequent immune challenges (Graham et al., 2006; Roque et al., 2014). Importantly, these shifts in immune function are known to persist for several years (Graham et al., 2006) and has the potential to persist into adulthood (Harry and Kraft, 2012; Delpech et al., 2016). This long-term reprogramming of the immune system has been suggested to underlie the emergence of depressive episodes in younger populations as subsequent stress exposures can produce augmented and poorly regulated physiological responses (Brown et al., 1977).

Sex

Over the last two decades special attention has been paid to understanding the putative contribution of sex, and more specifically gonadal hormones, to the consequences of stress exposure. This research interest was facilitated by several clinical reports that documented that women are more likely to be diagnosed with depression compared with men (Weissman and Klerman, 1992; Gallo et al., 1993; Kessler et al., 1993; Hankin et al., 1998). This two-fold increased risk is known to emerge at the onset of puberty, persists into adulthood (Kessler et al., 1993; Hankin et al., 1998; Nolen-Hoeksema, 2001), and ends following menopause (Kessler et al., 1993; Hankin et al., 1998) strongly suggesting that ovarian hormones may mediate this enhanced stress susceptibility in females. It is important to note that under non-stress conditions, ovarian hormones have consistently been suggested to confer protection because ovariectomy increases depressive-like behaviors (Li et al., 2014). However, when gonadally-intact and ovariectomized female mice are exposed to repeated stress, ovariectomy confers protection against stress-induced depressive-like behavior (LaPlant et al., 2009). Ovarian hormones, like androgens in males, exert control over a number of physiological systems including inflammation (Villa et al., 2016). This is of particular importance as women exhibit greater inflammatory-induced depressive behaviors following an acute endotoxin challenge compared to men (Moieni et al., 2015). Importantly, this ovarian hormone mediated control over inflammatory systems has also been reported in preclinical models demonstrating that female mice exhibit a greater number of microglia that also exhibit more reactive morphology in brain areas associated with emotional regulation (Schwarz et al., 2012). Moreover, when estrogen is administered *in vivo* and microglia are subsequently cultured, microglia with prior estrogen treatment are sensitized to LPS stimulation (Loram et al., 2012). However, it should be noted that the effect of estrogen on microglia have also been demonstrated to suppress cytokine release, but only when estrogen is applied *ex vivo* to microglial cells in culture (Dimayuga et al., 2005; Loram et al., 2012).

One of the most common forms of social stress conducted in the laboratory setting is the resident intruder paradigm of social defeat originally developed by Miczek (1979). Social defeat capitalizes on the protection and defense of territory. This model of social stress has proven to be very effective in males and readily produces anxiety- and depressive-like behaviors in the intruder rats (Wood et al., 2010, 2013, 2015; Chaijale et al., 2013; Patki et al., 2013; Finnell et al., 2017a). However, running social defeat in female rats can be difficult and requires either a lactating female resident (Jacobson-Pick et al., 2013) or modification of the male resident with DREADDs to induce heightened aggression via activation of the ventromedial hypothalamus (Takahashi et al., 2017). Recently a new modification to the resident intruder paradigm has also been conducted in which aggression by the male resident was induced following the application of male odorants to the female intruders (Harris et al., 2018). Exposure to this particular modality of social stress (i.e., defeat by a male resident) in

female rats has produced incongruent results (Haller et al., 1999; Huhman et al., 2003; Shimamoto et al., 2011; Trainor et al., 2011; Holly et al., 2012; Greenberg et al., 2013, 2015; Jacobson-Pick et al., 2013; Ver Hoeve et al., 2013; Takahashi et al., 2017; Harris et al., 2018). In contrast, findings from the Trainor lab have consistently demonstrated that female California mice display greater sensitivity to the behavioral and molecular consequences to social defeat stress compared with males (Trainor et al., 2011; Greenberg et al., 2013, 2015; Duque-Wilckens et al., 2018). These species dependent effects of social defeat stress in females may underscore the ethological relevance of this stress modality. Unlike female rats which demonstrate territorial aggression only during the lactation period, female California mice inherently demonstrate territorial aggression. These data suggest that the physical interaction of social defeat may be more ethologically relevant in female/male California mice and male rats compared with female rats. This assumption is further validated by studies demonstrating that female rats exhibit greater sensitivity to social isolation/instability compared with social defeat (Haller et al., 1999).

With this in mind, a new model of social stress has recently emerged that combines the olfactory, auditory and visual exposure of social defeat without requiring the physical interaction of defeat. Using this vicarious witness stress model originally developed for use in male mice by Warren et al. (2013), we have shown that intact female rats demonstrate greater sensitivity to the inflammatory, cardiovascular and behavioral consequences of witness stress exposure compared to ovariectomized female rats (Finnell et al., 2018). We have further demonstrated that this enhanced and prolonged behavioral and physiological sensitivity to the consequences of witness stress is not exhibited to the same extent in male rats (Finnell et al., 2017b). While this is still a relatively new model of stress, others have also been able to demonstrate similar behavioral sensitivity of intact female mice to this vicarious witness stress exposure (Iniguez et al., 2018), suggesting that female susceptibility to witness stress may be conserved across species. In humans, bearing witness to a major stressor is one type of event that can elicit post traumatic stress disorder (PTSD). Therefore, it should be noted that similar to findings in depressed patients, PTSD in the clinical setting is also associated with a significant shift in immune reactivity (reviewed in Segerstrom and Miller, 2004). Interestingly, this immune reactivity differs between men and women with men exhibiting a general under-expression of inflammatory related genes of collected $CD14^{+}$ monocytes while women exhibit an upregulation of pathways associated with inflammatory activation (Neylan et al., 2011). Several comprehensive reviews have recently been published regarding enhanced stress sensitivity and increased risk of mood disorders in females (Goel and Bale, 2009; Bangasser and Wicks, 2017; Bangasser and Wiersielis, 2018; Wickens et al., 2018). Moving forward it will be critical to further validate whether stress sensitive mechanisms in females are mediated in part by inflammatory processes.

Personality and Coping

It has long been recognized that there is wide variability in the way people process and assess stressful situations (Lupien et al., 2007). This may be driven by the individual's cognitive interpretation (Lupien et al., 2007; Nicolai et al., 2013) as well as the behavioral coping mechanism that is adopted during the stress exposure. In general, coping strategies are broadly classified into two categories termed passive and active. Passive coping strategies include avoidance, seeking excessive reassurance, withdrawal and substance abuse (Cambron et al., 2009; Cairns et al., 2014). In contrast, active coping strategies include problem solving, seeking support, exercising and engaging in adaptive processes (Cairns et al., 2014). It is understood that the coping response adopted by an individual will vary depending on the type and severity of the stress exposure. However, it has been suggested that individuals who more readily adopt active coping strategies are more likely to be resilient to the behavioral and physiological consequences of stress compared to those who more readily adopt passive coping strategies (Kendler et al., 1991). Importantly, coping responses have also been shown to play a large role in the inflammatory outcomes of stress. For example, individuals who more readily adopt passive coping strategies exhibit greater plasma concentrations of IL-6 following a 3 min simulated public speaking challenge compared with individuals that adopt active coping strategies (Carroll et al., 2011). Additionally, feelings of helplessness during stress exposure are associated with sensitized immune responses to a common allergen and promote greater release of IL-6 from stimulated primary blood leucocytes (Kiecolt-Glaser et al., 2009). These data suggest that feelings of helplessness or uncontrollability could promote sensitization of inflammatory pathways that can be amplified by stress exposure (Chen et al., 2009). Although it is impossible to truly assess the emotional state of an animal, a number of preclinical studies demonstrated that both coping (Koolhaas et al., 1999, 2007; Sih et al., 2004; Bell, 2007; Wood et al., 2015; Finnell and Wood, 2016) and stressor controllability (Gray and Cooney, 1982; Frank et al., 2007; Christianson et al., 2009; Arakawa et al., 2014) are large factors in the susceptibility for developing stress-induced behavioral and inflammatory dysfunction. Several recent reviews have also been published on the topic of stress coping and inflammatory outcomes (Maier and Watkins, 2005; Koolhaas et al., 2007; Wood, 2014; Finnell and Wood, 2016; Wood et al., 2017).

BRAIN AREAS ASSOCIATED WITH STRESS SUSCEPTIBILITY AND RESILIENCY

There are a number of brain regions that have been implicated in the emergence of stress-induced behavioral dysfunction that are discussed throughout this review. Several extensive reviews have been published on this topic, for example (McEwen and Gianaros, 2010). However, to highlight the importance of the brain regions described herein, we have included a brief overview

of the brain areas that are associated with social processing and stress responses.

Prefrontal Cortex

The prefrontal cortex works to integrate the social, emotional and cognitive aspects of behavior (Satpute and Lieberman, 2006). Dysfunction within the prefrontal cortex in humans has been associated with the emergence of socially inappropriate behaviors, apathy, inflexibility and isolation (Barrash et al., 2000). In addition to producing overall shifts in social behavior, the prefrontal cortex has also been implicated in stress-induced coping strategies (Robinson et al., 2015). Similar associations between prefrontal cortex activation and susceptibility to the consequences of stress have also been demonstrated in rodents. Utilizing chronic social defeat stress in mice, it was shown that individual susceptibility to the behavioral effects of chronic social defeat (i.e., social avoidance) was directly associated with the activity of the prefrontal cortex (Kumar et al., 2014). Moreover, Kumar et al. (2014) went on to demonstrate that prefrontal cortex reactivity during a pre-stress forced interaction test was predictive of individual stress susceptibility following chronic social defeat. In the context of emotional regulation and threat assessment, the prefrontal cortex serves as a top down inhibitory regulator of the amygdala and hypothalamus (Mujica-Parodi et al., 2017). Several clinical and preclinical studies have consistently reported that stress-induced behavioral deficits are often associated with dendritic atrophy, loss of synapses, and altered prefrontal cortex connectivity (Radley and Morrison, 2005; Banasr et al., 2007; Drevets et al., 2008; Radley et al., 2008; Ota et al., 2014). These morphological and physiological alterations of prefrontal cortex neurons may therefore result in disinhibition of downstream signaling targets including the amygdala.

Hippocampus

Although largely known for its role in declarative memory, the hippocampus has also been implicated in social and emotional episodic memories (Dolcos et al., 2017). Through its connectivity and bottom-up signaling with the amygdala, the hippocampus is responsible for the encoding and retrieval of emotionally laden memories (Dolcos et al., 2017). In addition, the hippocampus is also critical for the re-encoding and extinction of these memories. Exposure to chronic unpredictable restraint stress was shown to produce reductions in several hippocampal sub regions including CA1, CA3 and the dentage gyrus (Schoenfeld et al., 2017). Reductions of hippocampal volume in response to stress have been associated with both dendritic atrophy (Watanabe et al., 1992; Wood et al., 2004; Eiland and McEwen, 2012) and reduced neurogenesis (Simon et al., 2005; Jayatissa et al., 2006; Mitra et al., 2006; Schoenfeld et al., 2017). Interestingly, preclinical studies using social defeat in mice have indicated that defeat-induced reductions of neurogenesis within the hippocampus is associated with stress susceptibility (Tse et al., 2014) and mice demonstrating stress resiliency exhibited an increase of hippocampal neurogenesis by approximately 4% (Tse et al., 2014).

Amygdala

The amygdala is best known for its role in fear responses. For example animals with lesions of the amygdala exhibit a disinhibition of fear responses and a significant increase in prosocial behavior (Kluver and Bucy, 1997). Chronic stress is also known to produce significant structural and functional effects within the amygdala that are highly dependent upon the type and duration of the stressor (Wilson et al., 2015). Moreover, it is now well recognized that depression and anxiety are both associated with amygdala hyperactivity (Drevets, 2000; Sheline et al., 2001). While it is currently unclear how active and passive stress coping strategies are associated with amygdala activity, it has been shown using rodent models that resilient individuals exhibit a number of stress-induced adaptations that may inhibit over activation of the amygdala (Silveira Villarroel et al., 2018).

Bed Nucleus of the Stria Terminalis

Considered to be a part of the extended amygdala, the bed nucleus of the stria terminalis (BNST) is best known for its involvement in behaviors associated with social bonding (Coria-Avila et al., 2014), aggression (Nelson and Trainor, 2007), mating (Coria-Avila et al., 2014) and stress-induced cardiovascular function (Crestani et al., 2013; Oliveira et al., 2015). Interestingly, fMRI studies in humans have determined that the BNST is also involved in the generation of anticipatory anxiety to unpredictable noxious stimuli (Straube et al., 2007; Alvarez et al., 2011; Yassa et al., 2012). Although it is currently unclear how stress affects BNST function in humans, studies using rodent models have determined that exposure to stress results in enhanced BNST activation (Kollack-Walker et al., 1997; Martinez et al., 1998). It is also important to note that the BNST is a sexually dimorphic brain region that has been shown to play a critical role in the consequences of social defeat exposure in male and female monogamous California mice. Following social defeat exposure, Greenberg et al. (2013) showed that female California mice not only demonstrated greater social avoidance but also exhibited greater brain derived neurotrophic factor in the BNST compared to their defeated male counterparts.

Nucleus Accumbens

The Nucleus Accumbens (NAc) is largely studied in the field of addiction due to its role in motivation and reward. However, the NAc is quickly gaining attention in the field of stress and depression due to its potential involvement in the development of anhedonia (Di Chiara et al., 1999; Yadid et al., 2001). The NAc is predominantly inhibitory, releasing γ-aminobutylic acid (GABA) in the ventral tegmental area, thereby exerting control over cortical dopamine (Shirayama and Chaki, 2006). fMRI studies conducted in humans have shown that patients suffering from major depressive disorder exhibit altered activation of the NAc during reward anticipation and outcome compared to healthy controls (Misaki et al., 2016). Importantly, the NAc has also been implicated in coping behaviors. Use of active coping behaviors was associated with increases in NAc activity while passive coping was association with reductions in NAc activity (Levita et al., 2012).

SOURCES OF STRESS-INDUCED NEUROINFLAMMATION

While it is clear that neuroinflammatory processes may be a critical link in the pathogenesis of stress-related psychiatric disorders in certain subpopulations of patients, it is important to understand which stress sensitive processes are capable of promoting neuroinflammation. The two most likely mechanisms of increased neuroinflammation include stress-induced activation and sensitization (i.e., priming) of microglia and stress-induced disruption of the blood brain barrier (BBB; see **Figure 1**).

Microglial Activation and Priming

Microglia are considered to be highly adaptive cell types as they are capable of transitioning between pro-inflammatory (M1) and anti-inflammatory (M2) states. However, in response to stress a greater number of microglia exhibit the proinflammatory M1 phenotype (Tang et al., 2018). This change in morphology can be stimulated by activation of glucocorticoid receptors (GCRs; Ros-Bernal et al., 2011; Liu et al., 2016) found on the cell surface of microglia, suggesting that stress-induced release of cortisol (in humans) and corticosterone (in rodents) could promote this shift to an M1 microglial state. The involvement of M1 type microglia in stress-induced neuroinflammation has further been supported by studies utilizing the tetracycline analog minocycline. Minocycline, traditionally used as an antibiotic, is well documented to inhibit the polarization of microglia to an M1 proinflammatory phenotype (Kobayashi et al., 2013). Moreover, use of minocycline in conjunction with inescapable foot shock (Blandino et al., 2006) and chronic mild stress (Wang et al., 2018) have shown that inhibition of the M1 microglial phenotype, and subsequent suppression of proinflammatory cytokine release, protects against the development of stress-induced depressive- and anxiety-like responses in rodents. Notably, minocycline is now being evaluated as a putative treatment for bipolar depressive disorder in humans. A very recent clinical trial demonstrated that daily doses of minocycline was capable of producing anti-depressant effects in 90% of study participants (Murrough et al., 2018). While more information is required about the putative anti-inflammatory effects minocycline may have in these patients, these studies in combination provide clear evidence for the involvement of M1 microglia in the emergence of depressive symptomatology.

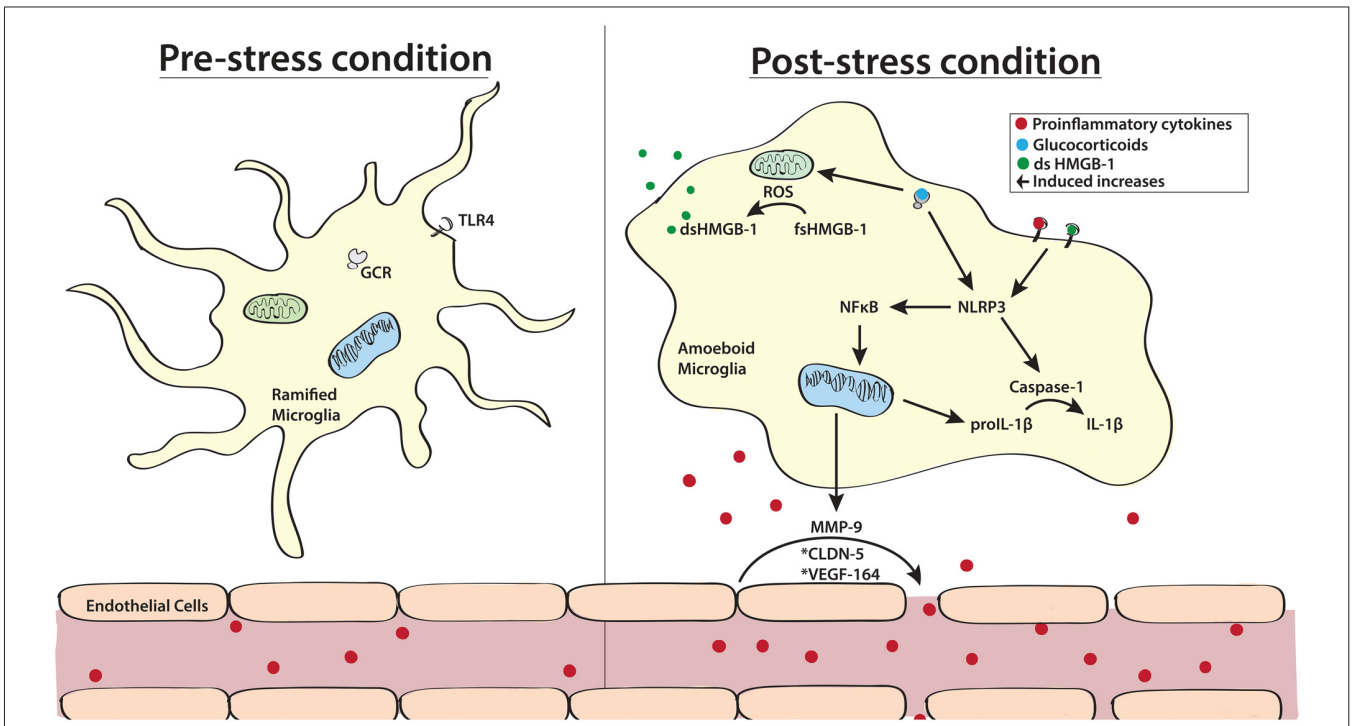

FIGURE 1 | Schematic highlighting key sources of stress-induced neuroinflammation. Stress exposure is known to promote shifts in microglial morphology from a highly ramified "resting" state to an ameboid M1 proinflammatory state. In addition to directly stimulating the release of cytokines, activation of microglial glucocorticoid receptors (GCRs) also results in priming of inflammatory responses. This process can occur directly through activation of the NLRP3 inflammasome or indirectly by promoting the release of reactive oxygen species (ROS) from mitochondria which results in the oxidation of high mobility group box -1 (HMGB-I). Once released, HMGB-1 and proinflammatory cytokines such as interleukin (IL)-1β can act on toll like receptor 4 (TLR 4) on the surface of microglia to further stimulate the NLRP3 signaling cascade. Another significant source of stress-induced neuroinflammation is the breakdown of the blood brain barrier (BBB). In pre-stress conditions endothelial cells tightly adhere to one another, blocking the flow of circulating cytokines to the brain. However, in response to stress exposure, tight junctions between these endothelial cells break down allowing for peripheral cytokines and inflammatory cells to penetrate into the brain. This process is known to be facilitated by plasma vascular endothelial growth factor (VEFG)-164, endothelial claudin-5 (CLDN-5) and microglia released matrix metalloproteinase-9 (MMP-9). *Designate non-neuronal and non-glial origins.

In addition to promoting the release of cytokines from microglia (Nair and Bonneau, 2006; Kreisel et al., 2014), stress is also capable of sensitizing microglia such that a subsequent stress or immune challenge produces a faster and more robust neuroinflammatory response (Frank et al., 2012, 2018; Fonken et al., 2016). Importantly, glucocorticoid signaling is one factor that has been shown to initiate this phase of stress-induced neuroinflammatory sensitization termed microglial priming. One potential mechanism by which this priming may occur is though the dysregulation of the danger, damage and disease signal high mobility group box-1 (HMGB-1). In response to stress, the membrane glycoprotein CD200 and its receptor (CD200R) exhibit significant down regulation at both the genomic and protein levels (Frank et al., 2018). Notably CD200R is almost exclusively expressed on microglia (Koning et al., 2009) and is known to regulate proinflammatory signaling by constitutively inhibiting myloid cells (Gorczynski, 2005). Loss of CD200 and CD200R in rats exposed to inescapable foot shock was further associated with enhancement of HMGB-1 and increased gene expression of IL-1β, tumor necrosis factor (TNF)-α and nuclear factor kappa (NFκ)B (Frank et al., 2018). This increased expression of proinflammatory genes by HMGB-1 has been directly linked to the activation of the nucleotide-binding oligomerization domain-like receptor (NLRP3) inflammasome (Weber et al., 2015). In addition to the noted effects on gene expression, HMGB-1 activation of the NLRP3 inflammasome can further potentiate proinflammatory signaling by enhancing the cleavage of proIL-1β to IL-1β via activation of caspase-1 (Yan et al., 2012). However, it is important to note that this proinflammatory capacity of HMGB-1 is strongly tied to the redox state of the protein. In its fully reduced state, HMGB-1 promotes chemotaxis but lacks the ability to promote proinflammatory signaling. Alternatively, the oxidized state of HMGB-1, designated by the formation of disulfide linkages, is capable of potentiating proinflammatory signaling as discussed above but lacks chemotactic abilities (Yang et al., 2012). Although the majority of studies assessing the involvement of HMGB-1 in microglial priming have come from studies using inescapable foot shock (Yang et al., 2012; Weber et al., 2015), chronic unpredictable stress (Franklin et al., 2018), and single prolonged stress (Lai et al., 2018), exposure to social stressors such as social defeat is known to enhance the intracellular concentration of reactive oxygen species (ROS; see section Oxidative Stress/Reactive Oxygen Species). Therefore, it is highly plausible that HMGB-1 may also contribute to the emergence of social stress-induced behavioral deficits.

These stress-induced alterations in the morphology and reactivity of microglia requires several hours to manifest and are evident for up to 72 h following the termination of stress, a time point at which peripheral cytokine responses are no longer detected (Tynan et al., 2010; Kopp et al., 2013; Deak et al., 2017). These data nicely parallel findings indicating that the development of depressive-like behaviors following a robust inflammatory challenge occurs over a period of several hours and persist well beyond 24 h (Capuron et al., 2002; Dantzer et al., 2008). Moreover, preclinical studies using social defeat and vicarious witness stress have demonstrated that repeated stress exposure is capable of enhancing resting neuroinflammation that persists for at least 5 days following the final stress exposure, a time at which depressive-like behavior is evident (Finnell et al., 2017a,b). Importantly these studies determined that despite elevations in neuroinflammation and depressive-like behavior 5 days following the final stress exposure, resting peripheral inflammation had returned to baseline comparable to non-stressed controls (Finnell et al., 2017a). The importance of central inflammation in the emergence of stress-induced depressive-like behavior has been further substantiated by studies outlining the effectiveness of centrally administered IL-1 receptor antagonist in inhibiting social defeat-induced depressive-like behavior (Wood et al., 2015). Similar antidepressant-like effects were demonstrated with the use of resveratrol, a natural anti-inflammatory. Importantly, these effects were only achieved by the highest dose, which was the only dose to effectively prevent the neuroinflammatory response to social defeat (Finnell et al., 2017a). These data strongly suggest that stress likely promotes the emergence of an M1 microglial phenotype which may directly underlie stress and inflammatory-induced behavioral dysfunction.

Blood Brain Barrier Disruption

While cells within the brain are robustly capable of producing a major source of neuroinflammation, cytokines circulating in the blood can also serve as a source to increase neuroinflammation. The BBB, in part a meshwork of specialized endothelial cells along blood vessels surrounding the brain, serves the purpose of regulating entry and export of cytokines (and other substances) between the peripheral circulation and the brain. In a healthy brain, cytokines are considered to be too large and hydrophilic to passively diffuse across the BBB (Banks, 2005). However, the IL-1 family, TNF and IL-6 exhibit distinct and saturable transport mechanisms to effectively pass from the blood to the brain (Banks et al., 1989, 1991). Moreover, pro-inflammatory cytokines can disrupt the integrity of the BBB (Muramatsu et al., 2012). As such, circulating inflammation may initiate a cascade that enhances the flow of inflammatory factors from the circulation into the brain, further exacerbating neuroinflammation. This concept was demonstrated in an elegant study that showed that microglia initiated the recruitment of IL-1β producing monocytes to the brain and stimulated brain endothelial IL-1R1 (McKim et al., 2018). This study went on to further demonstrate that microglial depletion prevented monocyte recruitment and inhibited the development of anxiety in socially defeated mice.

It is tempting to suggest that the link between diseases characterized by peripheral inflammation including cardiovascular disease, rheumatoid arthritis, etc., and the striking increased risk of major depression in these patients (Anda et al., 1993; Huffman et al., 2013; Marrie et al., 2017) may be driven by an impaired BBB and exaggerated neuroinflammation. In addition, social stress exposure, another risk factor for psychiatric disorders is recognized to increase the release of circulating proinflammatory cytokines in animals and humans (Pace et al., 2006; Hodes et al., 2014; Wood et al., 2015; Quinn et al., 2018). While circulating cytokine

levels typically return to baseline following cessation of a single acute social stressor (Cheng et al., 2015), preclinical models generating a stress-induced depressive-like phenotype achieved by repeated exposure to social defeat stress demonstrate persistent enhancement in peripheral inflammatory sensitivity (Hodes et al., 2014; Finnell et al., 2017a). In line with the deleterious role of pro-inflammatory cytokines on the integrity of the BBB, recent reports have identified the role of social stress on various factors known to disrupt the BBB. For example, male rats that demonstrate susceptibility to social defeat stress as evidenced by passive coping responses during social defeat and development of depressive-like behaviors, selectively demonstrated enhanced BBB permeability in the ventral hippocampus (Pearson-Leary et al., 2017) while the active coping resilient subset of rats did not. Moreover, administration of the proinflammatory cytokine vascular endothelial growth factor-164 increased permeability of the BBB and was shown to induce vulnerability in socially defeat rats (Pearson-Leary et al., 2017). Stress-induced BBB disruption has also been documented in a mouse model of social defeat, whereby the susceptible subset of male mice also demonstrated stress-induced suppression of claudin-5, an endothelial cell-specific tight junction protein, in the NAc and the hippocampus as compared with controls or the resilient subset of mice. Moreover, BBB permeability was also confirmed in the susceptible subset of mice (Menard et al., 2017). Importantly, these studies further established suppressed claudin-5 expression in post mortem tissue from the NAc of depressed patients. Taken together, disruption of the BBB is a likely susceptibility mechanism driving increased neuroinflammation and social stress-induced behavioral dysfunction in animals, and may contribute to psychopathology in humans.

Other proteins are likely targets for stress-induced increases in BBB permeability and include HMGB-1 and matrix metalloproteinase-9 (MMP-9). For example, HMGB-1 is upregulated by social defeat stress (Finnell et al., 2017b) and beyond its role in neuroinflammatory priming, is also involved in BBB dysfunction. This role for HMGB-1 is supported by studies demonstrating that administration of monoclonal antibody to HMGB-1 protects against ischemia-induced BBB disruption in rats (Zhang et al., 2011), and in humans anti-HMGB1 monoclonal antibody improves the BBB integrity of patients with Alzheimers disease (Festoff et al., 2016). Together these findings clearly define a role for HMGB-1 in BBB dysfunction that could precipitate stress-related psychiatric dysfunction. Furthermore, inflammatory factors including HMGB-1 (Qiu et al., 2010) also stimulate the release of MMP-9, a zymogen that breaks down the BBB, from infiltrating leukocytes and microglia to contribute to endothelial damage (Crocker et al., 2006) and BBB leakage (Seo et al., 2013). MMP-9 protein expression is elevated in peripheral tissues and serum by social defeat stress (Stelzhammer et al., 2015; Wu et al., 2015). While this has yet to be documented in the brain following social defeat, central MMP-9 has been shown to be regulated by fear learning (Ganguly et al., 2013) and lends support to the possibility that MMP-9 may be a putative target by which social stress could lead to BBB disruption.

CENTRAL MECHANISMS CONFERRING RISK OR RESILIENCE TO STRESS THAT ARE REGULATED BY NEUROINFLAMMATION

Acute stress is well recognized to stimulate the release of proinflammatory cytokines from microglia (Blandino et al., 2006, 2013) and repeated stress exposure is capable of producing enduring increases in neuroinflammation in stress sensitive brain regions (Voorhees et al., 2013; Wohleb et al., 2013; Wood et al., 2015; Finnell et al., 2017a,b). While evidence links an inflammatory state with a depressive phenotype, our understanding of exactly which neuromodulatory systems are acted upon by inflammatory cytokines and chemokines that serve to increase stress susceptibility is in its infancy. Several reviews have been published on the impact that neuroinflammation has on the metabolism of the neurotransmitters serotonin, dopamine and glutamate and therefore, while clearly relevant to the pathophysiology of depression, this topic will not be covered here (see Miller et al., 2013). Herein, we will focus on the potential role of neuroinflammation on mitochondrial dysfunction and oxidative stress as well as glutamate neurotransmission or excitotoxicity (see **Figure 2**).

Mitochondrial Dysfunction

Mitochondria play a critical role in cellular energy metabolism and supply the large energy demand required by the brain, especially under stressful conditions. The inner membrane of mitochondria houses the electron transport chain, which is made up of five protein complexes. Three of these respiratory chain complexes (I, III and IV) pump protons throughout the inner membrane generating the proton gradient that is ultimately responsible for synthesizing adenosine triphosphate (ATP) at complex V. Mitochondria are responsible for producing the vast majority of the ATP in neurons and in particular within presynaptic terminals mitochondrial ATP is required for synaptic ion homeostasis and phosphorylation (Mattson et al., 2008). There is mounting evidence that patients with psychiatric disorders demonstrate mitochondrial abnormalities at the functional level. For example, positron emission tomography studies of brain glucose metabolism have identified reduced glucose utilization in the brains of depressed patients (Videbech, 2000). Moreover, mitochondrial ATP production was also reduced in depressed patients (Gardner et al., 2003). While the cause of this mitochondrial dysfunction is not understood, it is noteworthy to consider the findings that proinflammatory cytokines can impair mitochondrial function. For example, physiologically relevant levels of TNF-α can induce mitochondrial dysfunction; low (post-stroke) levels of TNF-α rapidly reduce mitochondrial function as indicated by increased caspase 8 activity and a decrease in mitochondrial membrane potential (Doll et al., 2015). This effect was shown to signal through TNF-R1 selectively and highlights the role that proinflammatory cytokines may play in mitochondrial dysfunction. Beyond the capability of neuroinflammation to

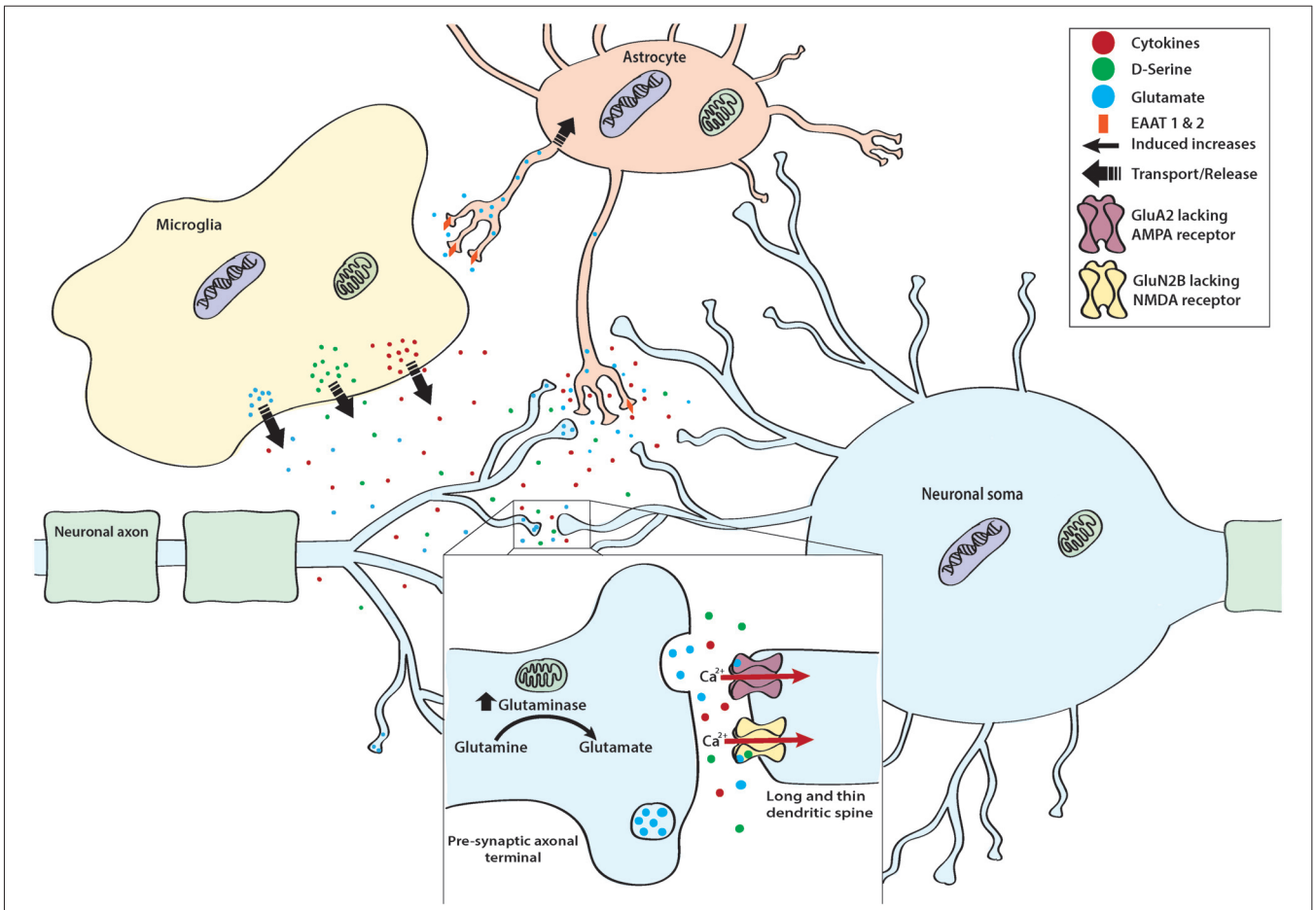

FIGURE 2 | Mechanisms of stress-induced cytotoxicity. In addition to releasing cytokines, MI type proinflammatory microglia release a variety of neurotransmitters, co-agonists, and neuromodulators such as glutamate and its co-agonist D-serine. Normally, excess glutamate is taken up by excitatory amino acid transporter (EAAT) 1 and 2 found on astrocytic processes. However, in proinflammatory conditions and in the presence of excess glutamate, EAAT 1 and 2 are down regulated, thereby resulting in excess glutamate within the synaptic cleft. Importantly, neurons also contribute to stress-induced enhancements of glutamatergic tone. This is thought to occur as stress exposure enhances mitochondrial glutaminase, the enzyme responsible for converting glutamine to glutamate. In addition to enhancing excitatory tone, stress also sensitizes neurons to the excitatory effect of glutamate. Specifically, stress promotes the expression of GluA2 lacking α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and GLUN2B lacking NMDA receptors. These receptor subtypes allow for calcium (Ca^{2+}) to freely pass into the cell thereby enhancing the depolarizing effect of glutamate. This cumulative increase in excitatory tone is particularly detrimental for dendritic spines that exhibit a long and thin morphology, as these spines are more sensitive to the degenerative effects induced by glutamatergic excitotoxicity.

induce mitochondrial dysfunction, it is interesting to note that reducing activity of mitochondria within microglia amplifies the NLRP3 inflammasome and IL-1β release (Sarkar et al., 2017). Taken together these studies demonstrate a striking relationship between neuroinflammation and mitochondrial dysfunction.

While no studies to date have directly evaluated the role that stress-induced release of proinflammatory cytokines has on mitochondrial function, it has been demonstrated in various stress paradigms that repeated stress exposure has dramatic effects on mitochondria. For example, chronic immobilization stress and chronic mild stress inhibit the activity of the respiratory chain complexes within the rat brain cortex (Madrigal et al., 2001; Rezin et al., 2008) and was shown to reduce hippocampal Na^+, K^+-ATPase activity (Gamaro et al., 2003). Moreover, chronic mild stress has been shown to reduce respiration rates of mitochondria located in the mouse hippocampus, cortex and hypothalamus (Gong et al., 2011). This study also confirmed that stress significantly impacted the ultrastructure of mitochondria (Gong et al., 2011), which are features of mitochondria in presynaptic neurons that have been coupled to changes in synaptic strength (Cserép et al., 2018). Moreover, there is evidence to suggest that distinct differences in mitochondrial function regulate an anxiety-like phenotype. For example, rats that exhibited high anxiety-like behavioral tendencies also demonstrated reduced expression of mitochondrial complex I and II proteins and decreased respiratory capacity and ATP (Hollis et al., 2015). Surprisingly, however, there is a paucity of studies evaluating the impact of social defeat stress on brain mitochondria and even further lack of studies determining whether the vast stress-induced changes in mitochondrial function are driven by stress-induced proinflammatory cytokines.

Oxidative Stress/Reactive Oxygen Species (ROS)

Active neurons exhibit high rates of oxygen consumption and as a result, produce large amounts of ROS (Halliwell, 1992). Mitochondria are the energy powerhouse of the cell and represent the largest source of ROS production in addition to monoamine oxidase and nitric oxide synthase. While ROS play a role in several critical neuronal functions such as neuronal plasticity and learning and memory (reviewed in: Massaad and Klann, 2011), the large amounts of ROS are tightly regulated by an antioxidant system. Under conditions where this system becomes unbalanced, a deleterious buildup of ROS is linked to stress-related psychiatric pathology in clinical studies and is demonstrated to occur in stress-related preclinical studies (de Oliveira et al., 2007; Salim et al., 2010, 2011; Lindqvist et al., 2017). Because mitochondria play a critical role in the production and metabolism of ROS, mitochondrial dysfunction is directly related to increased oxidative stress (Mattson et al., 2008). In line with evidence discussed above that TNF-α reduces mitochondrial function, ROS are also dose dependently increased by treatment with either TNF-α or IL-6 (Rochfort et al., 2014). Social defeat stress has also been shown to induce ROS in stress-related brain regions, and moreover ROS have been shown to play a permissive role in the anxiety-like behavior following social defeat in rats (Solanki et al., 2017). Interestingly, rats demonstrating a high anxiety-like phenotype also exhibit increased ROS production within the NAc (Hollis et al., 2015). Finally, lending evidence to the role for ROS in the pathogenesis of psychiatric disorders in humans, depressed patients not only exhibited elevated markers of inflammation and the oxidative stress marker F2-isoprostanes, but compared to individuals who readily respond to antidepressants, non-responders had higher levels of both oxidative stress markers and inflammation (Strawbridge et al., 2015; Vaváková et al., 2015; Lindqvist et al., 2017). Taken together, it is clear that proinflammatory cytokines are capable of shifting the balance of ROS production/elimination from a healthy balance towards maladaptive. However, it is yet to be determined whether stress-induced ROS and subsequent anxiety- and depressive-like behavior is initiated by proinflammatory cytokines and chemokines.

Glutamate Neurotransmission and Excitotoxicity

The involvement of glutamate has also become an area of interest in the etiology of depression. For example, heightened excitability of hippocampal neurons may underlie the loss of glutamatergic pyramidal neurons in depressed patients (Rajkowska et al., 2005) and evidence from human postmortem tissue has identified alterations in excitatory amino acid transporters (EAATs) 1 and 2 and glutamine synthetase (Rajkowska and Stockmeier, 2013). Moreover, it has been shown that ketamine, a noncompetitive NMDA antagonist (Anis et al., 1983), is capable of producing long lasting antidepressant effects (Berman et al., 2000) even in patients that demonstrate resistance to traditional antidepressant therapies (Zarate et al., 2006). Importantly, the inhibitory action of ketamine requires the presence of open NMDA channels (MacDonald et al., 1987) and can remain bound to NMDA receptors even after the channels have closed (Huettner and Bean, 1988), providing pharmacological validity to these prolonged treatment effects. Several preclinical models have demonstrated that exposure to stress can result in abnormalities in glutamate signaling. For example, 8 weeks of social isolation has been shown to enhance the expression of both NR2A and NR2B subunits within the hippocampus (Chang et al., 2015). Stress-induced increases in these NMDA receptor subunits within the hippocampus are known to not only enhance the intensity of excitatory postsynaptic potentials (Chang et al., 2015) but are also associated with the emergence of aggression, anxiety- and depressive-like behaviors in rodents (Costa-Nunes et al., 2014; Chang et al., 2015). However, it is important to note that these alterations in NMDA receptor subunits following stress exposure are brain region specific. Within the NAc, mice exposed to chronic social defeat that also demonstrate behavioral susceptibility, exhibit long term reductions of NR2B subunit (Jiang et al., 2013). The loss of NR2B subunits significantly impacted the synaptic function of NAc neurons by promoting an increase in long-term depression (Jiang et al., 2013). Interestingly, this study went on to determine that treatment with Fluoxetine, a selective serotonin re-uptake inhibitor, was capable of reversing the effects of defeat stress in susceptible mice such that the molecular profiles within the NAc were nearly identical to mice demonstrating resilience to the effects of social defeat (Jiang et al., 2013).

Stress-induced alterations of NMDA receptors are not the only putative source of glutamatergic excitotoxicity in the brain. For example, unpredictable stress exposure has been documented to produce similar alterations in the subunit composition of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors such that stress exposed rodents demonstrated greater expression of GluR1 subunits (Hubert et al., 2014). Moreover, exposure to the unpredictable stress paradigm resulted in a shift in AMPA receptor distribution such that a greater number of AMPA receptors were found on dendritic spines (Hubert et al., 2014).This seemingly minor shift is well known to produce functionally relevant alterations in neuronal signaling. AMPA receptors which express the GluR2 subunit are impermeable to extracellular Ca^{2+} due to an arginine block. Therefore, loss of GluR2 subunits enhances the signaling strength of AMPA receptors by enhancing the magnitude of the elicited depolarization following AMPA receptor stimulation (Isaac et al., 2007). Taken together, these findings suggest that exposure to unpredictable stress may result in significant remodeling of dendritic spines to vastly increase their sensitivity to excitatory stimuli. These effects, similar to those demonstrated in microglia, require at least 24 h following the cessation of stress to become evident suggesting that these alterations are largely driven by alterations in gene expression (Nasca et al., 2015) and are not associated with the immediate stress response. Interestingly, GluR2 subunits are also known to shift across the lifespan. In rodents it has been shown that GluR2 steadily increases from birth until adulthood. However, this composition of AMPA

receptors does not remain stable and does decrease such that 70-week-old rodents exhibit significant decline in both protein and mRNA for the GluR2 subunits within the hippocampus (Pandey et al., 2015). While it is unclear if inflammatory senescence and enhanced inflammatory reactivity is associated with this shift in GluR2 subunits, these data suggest that a natural loss of GluR2 may contribute to the enhanced risk of mood disorders in aging populations.

A number of studies have directly implicated neuroinflammation and microglial processes in the emergence of glutamatergic excitotoxicity (Faust et al., 2010; Diamond and Volpe, 2012). Most directly, glutamate can be released from activated microglia (Barger et al., 2007) or neurons following stimulation with cytokines such as IL-1β in a dose dependent manner (Zhu et al., 2006). In addition, cytokines released by neighboring microglia are capable of acting upon neurons to increase neuronal glutaminase (Ye et al., 2013), a mitochondrial enzyme responsible for the conversion of glutamine to glutamate (Zhao et al., 2012). Importantly, TNF-α-induced increases in glutaminase have been tied to the induction of ROS (Wang K. et al., 2017), demonstrating the functional overlap that exists in these stress and inflammatory sensitive systems. In addition to stimulating the release of glutamate, microglia can actively synthesize and release D-serine (Wu et al., 2004). D-serine is a co-agonist for NMDA receptors and strikingly exhibits a three-fold greater affinity for the receptor compared with glycine (Matsui et al., 1995). Several studies have demonstrated that exposure to social defeat stress in mice is capable of enhancing D-serine that is associated with anxiety- and depressive-like behaviors (Wang J. et al., 2017; Dong et al., 2018). Moreover, genetic deletion of D-serine was capable of conferring resilience to mice exposed to chronic social defeat (Dong et al., 2018). While it is currently unclear if these defeat-induced increases in D-serine are driven by defeat-induced proinflammatory cytokines or activation of microglia, it has been shown that administration of nonsteroidal anti-inflammatories such as mefenamic acid (Armagan et al., 2012a,b), acetaminophen, and naproxen (Armagan et al., 2012a) are capable of inhibiting D-serine.

Importantly, the role of stress and inflammation in glutamatergic excitotoxicity extends beyond glutamate receptors and their ligands. A number of studies have further demonstrated microglial involvement in glutamate accumulation in the extracellular space. Specifically, microglial stimulation with IL-1β (Ye et al., 2013) or TNF-α (Takeuchi et al., 2006; Ye et al., 2013) promotes the release of microglial glutamate. Under normal resting non-stress conditions, the brain has a number of mechanisms in place to manage excess synaptic glutamate. One such method is astrocyte mediated uptake via EAAT1 and EAAT2 in humans and glutamate–aspartate transporter (GLAST) and glutamate transporter 1 (GLT1) in rodents (Bezzi et al., 2004; Furuta et al., 2005). However, this protective mechanism has been shown to fail in instances where glutamate accumulation resulted from stimulation of microglia. Specifically, accumulation of glutamate in astrocytes results in a compensatory downregulation of EAAT1 (Takaki et al., 2012). Although preclinical studies assessing the role of GLAST and GLT1 in social stress-induced behavioral dysfunction has not been assessed, clinical studies have demonstrated altered expression of EAAT1 and 2 within brains of depressed patients (Miguel-Hidalgo et al., 2010; Rajkowska and Stockmeier, 2013). Together these data indicate that cytokine activation of microglia may result in a complex dysregulation of glutamate neuronal transmission by both enhancing local glutamate synthesis, stimulating glutamate release, and indirectly resulting in a downregulation of receptors involved in the maintenance of extra synaptic glutamate.

Remodeling of Excitatory Synaptic Terminals

In addition to modifying the release, synthesis and uptake of glutamate, stress and inflammation are known to alter the structure of excitatory synaptic terminals. Specifically, it has been shown that chronic stress results in the loss of dendritic spines in areas such as the prefrontal cortex (Goldwater et al., 2009). This loss of spines is directly associated with the emergence of anxiety- and depressive-like behaviors (Qiao et al., 2016). Stress has further been postulated to contribute to these effects by modulating a number of factors including the synthesis and release of MMP-9. In addition to promoting disruptions in the BBB (see "Blood Brain Barrier Disruption" section), MMP-9 is also involved in synaptic plasticity and remodeling of dendritic spines (Wang et al., 2008). In the presence of MMP-9, dendritic spines reshape from a short and round to a long and thin morphology (Michaluk et al., 2011). These long and thin spines are suggested to be less effective in conducting excitatory signals as they restrict Ca^{2+} flow (Ebrahimi and Okabe, 2014). Moreover, the thin and elongated spines also demonstrate greater vulnerability to the damaging cellular consequences of stress exposure (Radley et al., 2008; Bloss et al., 2011). In this manner, MMP-9-induced remodeling of dendritic spines may reduce neuronal excitability and promote the loss of dendritic spines. While clinical studies documenting the role of MMP-9 in the emergence of stress-induced depression are lacking, preclinical studies have demonstrated that exposure to social defeat results in elevations of the cytokine IL-1α and MMP-9. Importantly, these findings were most pronounced in susceptible rodents (Stelzhammer et al., 2015). These effects of social defeat on dendritic spines is not limited to MMP-9. Within the NAc inhibition of κB kinase (IκK) has also been shown to promote the formation of long and thin spines in animals exposed to social defeat (Christoffel et al., 2012). Moreover, this study found a trend to suggest that a greater number of long and thin spines was negatively associated with social interaction which could be reversed by inhibiting IκK (Christoffel et al., 2012). This same group later showed that chronic exposure to social defeat was also associated with an increase in the number of immature stubby spines in the NAc (Christoffel et al., 2015). In accordance with their previous findings, a larger number of stubby spines was associated with the emergence of social avoidance (Christoffel et al., 2015).

In the developing brain, microglia are well known to contribute to the remodeling of neuronal synapses through a

process termed synaptic pruning (reviewed in Lenz and Nelson, 2018). Synaptic pruning has been described as a phagocytic event where immature and highly active synapses are permanently removed. It was originally suggested that this occurred via microglial engulfment of dendritic spines. However, a study by Weinhard et al. (2018) demonstrated that although microglia did contact dendritic spines, they did not completely engulf the dendritic spines for elimination. Instead it was found that microglia participate in a process termed trogocytosis in which only a small portion of the dendritic spine is phagocytosed (Weinhard et al., 2018). This process of trogocytosis also stimulates the formation of new long and thin filopodia shaped spines (Weinhard et al., 2018). While studies determining the involvement of microglial pruning in the consequences of stress exposure is unknown, it is probable that similar phagocytic processes could occur as a consequence of stress exposure.

CONCLUSION

Prospective studies have clearly linked inflammatory related disorders with increased risk of depression. Moreover, several clinical studies support the notion that neuroinflammation is associated with depressive symptomatology. However, our understanding of the mechanisms that are impacted by neuroinflammation, especially in the context of social stressors is at its infancy. Gaining a better understanding of neuroinflammatory-mediated adaptations that occur during stress and are capable of producing psychopathology will be a great advance in understanding the role of neuroinflammation in the etiology of depressive and anxiety disorders. Beyond the recognized effects of inflammatory cytokines on neurotransmitter and neuropeptide systems, inflammation may likely regulate susceptibility to social stress by altering the BBB, sensitizing microglia, producing mitochondrial dysfunction and oxidative stress as well as contributing to glutamate toxicity. This review highlights these cytokine-sensitive mechanisms that are favorably positioned to contribute to pathology, yet in many cases their direct regulation by inflammatory cytokines in the context of social stress has not been determined and will represent a great advance to the etiology of stress-induced psychiatric disorders.

AUTHOR CONTRIBUTIONS

SW researched and wrote a considerable amount of the review article (2.5–3 of the 5 sections) and edited the rest of the review written by JF. JF researched and wrote a large amount of this review article (2.5 of the 5 sections), and revised the document as suggested.

FUNDING

The authors were supported by research funding from the National Institute of Mental Health (1R01MH113892-01A1 to SW), the American Heart Association (15SDG22430017 to SW, 17PRE33670106 to JF), the Veterans Administration (I21 BX002085 to SW), and the Brain Behavior Research Foundation NARSAD Young Investigator Grant (26809 to SW).

REFERENCES

Al-Harbi, K. S. (2012). Treatment-resistant depression: therapeutic trends, challenges and future directions. *Patient Prefer. Adherence* 6, 369–388. doi: 10.2147/PPA.s29716

Allen, A. P., Kennedy, P. J., Dockray, S., Cryan, J. F., Dinan, T. G., and Clarke, G. (2017). The trier social stress test: principles and practice. *Neurobiol. Stress* 6, 113–126. doi: 10.1016/j.ynstr.2016.11.001

Almeida, D. M. (2005). Resilience and vulnerability to daily stressors assessed via diary methods. *Curr. Dir. Psychol. Sci.* 14, 64–68. doi: 10.1111/j.0963-7214.2005.00336.x

Alvarez, R. P., Chen, G., Bodurka, J., Kaplan, R., and Grillon, C. (2011). Phasic and sustained fear in humans elicits distinct patterns of brain activity. *Neuroimage* 55, 389–400. doi: 10.1016/j.neuroimage.2010.11.057

Anda, R., Williamson, D., Jones, D., Macera, C., Eaker, E., Glassman, A., et al. (1993). Depressed affect, hopelessness and the risk of ischemic heart disease in a cohort of U.S. adults. *Epidemiology* 4, 285–294. doi: 10.1097/00001648-199307000-00003

Anis, N. A., Berry, S. C., Burton, N. R., and Lodge, D. (1983). The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate. *Br. J. Pharmacol.* 79, 565–575. doi: 10.1111/j.1476-5381.1983.tb11031.x

Arakawa, K., Arakawa, H., Hueston, C. M., and Deak, T. (2014). Effects of the estrous cycle and ovarian hormones on central expression of interleukin-1 evoked by stress in female rats. *Neuroendocrinology* 100, 162–177. doi: 10.1159/000368606

Armagan, G., Kanit, L., and Yalcin, A. (2012a). Effects of non-steroidal antiinflammatory drugs on D-serine-induced oxidative stress *in vitro*. *Drug Chem. Toxicol.* 35, 393–398. doi: 10.3109/01480545.2011.633086

Armagan, G., Turunc, E., Kanit, L., and Yalcin, A. (2012b). Neuroprotection by mefenamic acid against D-serine: involvement of oxidative stress, inflammation and apoptosis. *Free Radic. Res.* 46, 726–739. doi: 10.3109/10715762.2012.669836

Badoer, E. (2010). Microglia: activation in acute and chronic inflammatory states and in response to cardiovascular dysfunction. *Int. J. Biochem. Cell Biol.* 42, 1580–1585. doi: 10.1016/j.biocel.2010.07.005

Banasr, M., Valentine, G. W., Li, X.-Y., Gourley, S. L., Taylor, J. R., and Duman, R. S. (2007). Chronic unpredictable stress decreases cell proliferation in the cerebral cortex of the adult rat. *Biol. Psychiatry* 62, 496–504. doi: 10.1016/j.biopsych.2007.02.006

Bangasser, D. A., and Wicks, B. (2017). Sex-specific mechanisms for responding to stress. *J. Neurosci. Res.* 95, 75–82. doi: 10.1002/jnr.23812

Bangasser, D. A., and Wiersielis, K. R. (2018). Sex differences in stress responses: a critical role for corticotropin-releasing factor. *Hormones* 17, 5–13. doi: 10.1007/s42000-018-0002-z

Banks, W. A. (2005). Blood-brain barrier transport of cytokines: a mechanism for neuropathology. *Curr. Pharm. Des.* 11, 973–984. doi: 10.2174/1381612053381684

Banks, W. A., Kastin, A. J., and Durham, D. A. (1989). Bidirectional transport of interleukin-1 alpha across the blood-brain barrier. *Brain Res. Bull.* 23, 433–437. doi: 10.1016/0361-9230(89)90185-8

Banks, W. A., Ortiz, L., Plotkin, S. R., and Kastin, A. J. (1991). Human interleukin (IL) 1 alpha, murine IL-1 alpha and murine IL-1 beta are transported from blood to brain in the mouse by a shared saturable mechanism. *J. Pharmacol. Exp. Ther.* 259, 988–996.

Barger, S. W., Goodwin, M. E., Porter, M. M., and Beggs, M. L. (2007). Glutamate release from activated microglia requires the oxidative burst and lipid peroxidation. *J. Neurochem.* 101, 1205–1213. doi: 10.1111/j.1471-4159.2007.04487.x

Barrash, J., Tranel, D., and Anderson, S. W. (2000). Acquired personality disturbances associated with bilateral damage to the ventromedial prefrontal region. *Dev. Neuropsychol.* 18, 355–381. doi: 10.1207/s1532694205 Barrash

Barrientos, R. M., Kitt, M. M., Watkins, L. R., and Maier, S. F. (2015). Neuroinflammation in the normal aging hippocampus. *Neuroscience* 309, 84–99. doi: 10.1016/j.neuroscience.2015.03.007

Bath, K. G., Manzano-Nieves, G., and Goodwill, H. (2016). Early life stress accelerates behavioral and neural maturation of the hippocampus in male mice. *Horm. Behav.* 82, 64–71. doi: 10.1016/j.yhbeh.2016.04.010

Bell, A. M. (2007). Evolutionary biology: animal personalities. *Nature* 447, 539–540. doi: 10.1038/447539a

Berman, R. M., Cappiello, A., Anand, A., Oren, D. A., Heninger, G. R., Charney, D. S., et al. (2000). Antidepressant effects of ketamine in depressed patients. *Biol. Psychiatry* 47, 351–354. doi: 10.1016/s0006-3223(99)00230-9

Bezzi, P., Gundersen, V., Galbete, J. L., Seifert, G., Steinhäuser, C., Pilati, E., et al. (2004). Astrocytes contain a vesicular compartment that is competent for regulated exocytosis of glutamate. *Nat. Neurosci.* 7, 613–620. doi: 10.1038/nn1246

Blandino, P. Jr., Barnum, C. J., and Deak, T. (2006). The involvement of norepinephrine and microglia in hypothalamic and splenic IL-1β responses to stress. *J. Neuroimmunol.* 173, 87–95. doi: 10.1016/j.jneuroim.2005.11.021

Blandino, P. Jr., Hueston, C. M., Barnum, C. J., Bishop, C., and Deak, T. (2013). The impact of ventral noradrenergic bundle lesions on increased IL-1 in the PVN and hormonal responses to stress in male sprague dawley rats. *Endocrinology* 154, 2489–2500. doi: 10.1210/en.2013-1075

Bloss, E. B., Janssen, W. G., Ohm, D. T., Yuk, F. J., Wadsworth, S., Saardi, K. M., et al. (2011). Evidence for reduced experience-dependent dendritic spine plasticity in the aging prefrontal cortex. *J. Neurosci.* 31, 7831–7839. doi: 10.1523/JNEUROSCI.0839-11.2011

Brites, D., and Fernandes, A. (2015). Neuroinflammation and Depression: microglia activation, extracellular microvesicles and microRNA dysregulation. *Front. Cell. Neurosci.* 9:476. doi: 10.3389/fncel.2015.00476

Brown, G. W., Harris, T., and Copeland, J. R. (1977). Depression and loss. *Br. J. Psychiatry* 130, 1–18. doi: 10.1192/bjp.130.1.1

Cairns, K. E., Yap, M. B., Pilkington, P. D., and Jorm, A. F. (2014). Risk and protective factors for depression that adolescents can modify: a systematic review and meta-analysis of longitudinal studies. *J. Affect. Disord.* 169, 61–75. doi: 10.1016/j.jad.2014.08.006

Cambron, J. M., Acitelli, L. K., and Pettit, J. W. (2009). Explaining gender differences in depression: an interpersonal contingent self-esteem perspective. *Sex Roles* 61, 751–761. doi: 10.1007/s11199-009-9616-6

Capitanio, J. P., and Lerche, N. W. (1991). Psychosocial factors and disease progression in simian AIDS: a preliminary report. *AIDS* 5, 1103–1106. doi: 10.1097/00002030-199109000-00007

Capuron, L., Ravaud, A., Neveu, P. J., Miller, A. H., Maes, M., and Dantzer, R. (2002). Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. *Mol. Psychiatry* 7, 468–473. doi: 10.1038/sj.mp.4000995

Carroll, J. E., Low, C. A., Prather, A. A., Cohen, S., Fury, J. M., Ross, D. C., et al. (2011). Negative affective responses to a speech task predict changes in interleukin (IL)-6. *Brain Behav. Immun.* 25, 232–238. doi: 10.1016/j.bbi.2010.09.024

Chaijale, N. N., Curtis, A. L., Wood, S. K., Zhang, X.-Y., Bhatnagar, S., Reyes, B. A. S., et al. (2013). Social Stress engages opioid regulation of locus coeruleus norepinephrine neurons and induces a state of cellular and physical opiate dependence. *Neuropsychopharmacology* 38, 1833–1843. doi: 10.1038/npp.2013.117

Chang, C.-H., Hsiao, Y.-H., Chen, Y.-W., Yu, Y.-J., and Gean, P.-W. (2015). Social isolation-induced increase in NMDA receptors in the hippocampus exacerbates emotional dysregulation in mice. *Hippocampus* 25, 474–485. doi: 10.1002/hipo.22384

Chen, E., Miller, G. E., Walker, H. A., Arevalo, J. M., Sung, C. Y., and Cole, S. W. (2009). Genome-wide transcriptional profiling linked to social class in asthma. *Thorax* 64, 38–43. doi: 10.1136/thx.2007.095091

Cheng, Y., Jope, R. S., and Beurel, E. (2015). A pre-conditioning stress accelerates increases in mouse plasma inflammatory cytokines induced by stress. *BMC Neurosci.* 16:31. doi: 10.1186/s12868-015-0169-z

Christianson, J. P., Thompson, B. M., Watkins, L. R., and Maier, S. F. (2009). Medial prefrontal cortical activation modulates the impact of controllable and uncontrollable stressor exposure on a social exploration test of anxiety in the rat. *Stress* 12, 445–450. doi: 10.1080/10253890802510302

Christoffel, D. J., Golden, S. A., Heshmati, M., Graham, A., Birnbaum, S., Neve, R. L., et al. (2012). Effects of inhibitor of κB kinase activity in the nucleus accumbens on emotional behavior. *Neuropsychopharmacology* 37, 2615–2623. doi: 10.1038/npp.2012.121

Christoffel, D. J., Golden, S. A., Walsh, J. J., Guise, K. G., Heshmati, M., Friedman, A. K., et al. (2015). Excitatory transmission at thalamo-striatal synapses mediates susceptibility to social stress. *Nat. Neurosci.* 18, 962–964. doi: 10.1038/nn.4034

Coria-Avila, G. A., Manzo, J., Garcia, L. I., Carrillo, P., Miquel, M., and Pfaus, J. G. (2014). Neurobiology of social attachments. *Neurosci. Biobehav. Rev.* 43, 173–182. doi: 10.1016/j.neubiorev.2014.04.004

Costa-Nunes, J., Zubareva, O., Araújo-Correia, M., Valença, A., Schroeter, C. A., Pawluski, J. L., et al. (2014). Altered emotionality, hippocampus-dependent performance and expression of NMDA receptor subunit mRNAs in chronically stressed mice. *Stress* 17, 108–116. doi: 10.3109/10253890.2013.872619

Crestani, C. C., Alves, F. H., Gomes, F. V., Resstel, L. B., Correa, F. M., and Herman, J. P. (2013). Mechanisms in the bed nucleus of the stria terminalis involved in control of autonomic and neuroendocrine functions: a review. *Curr. Neuropharmacol.* 11, 141–159. doi: 10.2174/1570159X11311020002

Crocker, S. J., Milner, R., Pham-Mitchell, N., and Campbell, I. L. (2006). Cell and agonist-specific regulation of genes for matrix metalloproteinases and their tissue inhibitors by primary glial cells. *J. Neurochem.* 98, 812–823. doi: 10.1111/j.1471-4159.2006.03927.x

Cserép, C., Pósfai, B., Schwarcz, A. D., and Dénes, A. (2018). Mitochondrial ultrastructure is coupled to synaptic performance at axonal release sites. *eNeuro* 5:ENEURO.0390-17.2018. doi: 10.1523/ENEURO.0390-17.2018

Cuffel, B., Wamboldt, M., Borish, L., Kennedy, S., and Crystal-Peters, J. (1999). Economic consequences of comorbid depression, anxiety and allergic rhinitis. *Psychosomatics* 40, 491–496. doi: 10.1016/s0033-3182(99)71187-4

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., and Kelley, K. W. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat. Rev. Neurosci.* 9, 46–56. doi: 10.1038/nrn2297

Deak, T., Kudinova, A., Lovelock, D. F., Gibb, B. E., and Hennessy, M. B. (2017). A multispecies approach for understanding neuroimmune mechanisms of stress. *Dialogues Clin. Neurosci.* 19, 37–53.

Delpech, J.-C., Wei, L., Hao, J., Yu, X., Madore, C., Butovsky, O., et al. (2016). Early life stress perturbs the maturation of microglia in the developing hippocampus. *Brain Behav. Immun.* 57, 79–93. doi: 10.1016/j.bbi.2016.06.006

de Oliveira, M. R., Silvestrin, R. B., Mello, E. S. T., and Moreira, J. C. (2007). Oxidative stress in the hippocampus, anxiety-like behavior and decreased locomotory and exploratory activity of adult rats: effects of sub acute vitamin A supplementation at therapeutic doses. *Neurotoxicology* 28, 1191–1199. doi: 10.1016/j.neuro.2007.07.008

Denicoff, K. D., Rubinow, D. R., Papa, M. Z., Simpson, C., Seipp, C. A., Lotze, M. T., et al. (1987). The neuropsychiatric effects of treatment with interleukin-2 and lymphokine-activated killer cells. *Ann. Intern. Med.* 107, 293–300. doi: 10.7326/0003-4819-107-2-293

Devorak, J., Torres-Platas, S. G., Davoli, M. A., Prud'homme, J., Turecki, G., and Mechawar, N. (2015). Cellular and molecular inflammatory profile of the choroid plexus in depression and suicide. *Front. Psychiatry* 6:138. doi: 10.3389/fpsyt.2015.00138

Di Chiara, G., Loddo, P., and Tanda, G. (1999). Reciprocal changes in prefrontal and limbic dopamine responsiveness to aversive and rewarding stimuli after chronic mild stress: implications for the psychobiology of depression. *Biol. Psychiatry* 46, 1624–1633. doi: 10.1016/s0006-3223(99)00236-x

Diamond, B., and Volpe, B. T. (2012). A model for lupus brain disease. *Immunol. Rev.* 248, 56–67. doi: 10.1111/j.1600-065X.2012.01137.x

Dickerson, S. S., Gable, S. L., Irwin, M. R., Aziz, N., and Kemeny, M. E. (2009). Social-evaluative threat and proinflammatory cytokine regulation: *an experimental laboratory investigation*. *Psychol. Sci.* 20, 1237–1244. doi: 10.1111/j.1467-9280.2009.02437.x

Dimayuga, F. O., Reed, J. L., Carnero, G. A., Wang, C., Dimayuga, E. R., Dimayuga, V. M., et al. (2005). Estrogen and brain inflammation: effects on microglial expression of MHC, costimulatory molecules and cytokines. *J. Neuroimmunol.* 161, 123–136. doi: 10.1016/j.jneuroim.2004.12.016

Dolcos, F., Katsumi, Y., Weymar, M., Moore, M., Tsukiura, T., and Dolcos, S. (2017). Emerging directions in emotional episodic memory. *Front. Psychol.* 8:1867. doi: 10.3389/fpsyg.2017.01867

Doll, D. N., Rellick, S. L., Barr, T. L., Ren, X., and Simpkins, J. W. (2015). Rapid mitochondrial dysfunction mediates TNF-alpha-induced neurotoxicity. *J. Neurochem.* 132, 443–451. doi: 10.1111/jnc.13008

Dong, C., Zhang, J. C., Ren, Q., Ma, M., Qu, Y., Zhang, K., et al. (2018). Deletion of serine racemase confers D-serine -dependent resilience to chronic social defeat stress. *Neurochem. Int.* 116, 43–51. doi: 10.1016/j.neuint.2018.03.008

Drevets, W. C. (2000). Neuroimaging studies of mood disorders. *Biol. Psychiatry* 48, 813–829. doi: 10.1016/S0006-3223(00)01020-9

Drevets, W. C., Price, J. L., and Furey, M. L. (2008). Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Struct. Funct.* 213, 93–118. doi: 10.1007/s00429-008-0189-x

Duque-Wilckens, N., Steinman, M. Q., Busnelli, M., Chini, B., Yokoyama, S., Pham, M., et al. (2018). Oxytocin receptors in the anteromedial bed nucleus of the stria terminalis promote stress-induced social avoidance in female california mice. *Biol. Psychiatry* 83, 203–213. doi: 10.1016/j.biopsych.2017.08.024

Ebrahimi, S., and Okabe, S. (2014). Structural dynamics of dendritic spines: molecular composition, geometry and functional regulation. *Biochim. Biophys. Acta* 1838, 2391–2398. doi: 10.1016/j.bbamem.2014.06.002

Eiland, L., and McEwen, B. S. (2012). Early life stress followed by subsequent adult chronic stress potentiates anxiety and blunts hippocampal structural remodeling. *Hippocampus* 22, 82–91. doi: 10.1002/hipo.20862

Faust, T. W., Chang, E. H., Kowal, C., Berlin, R., Gazaryan, I. G., Bertini, E., et al. (2010). Neurotoxic lupus autoantibodies alter brain function through two distinct mechanisms. *Proc. Natl. Acad. Sci. U S A* 107, 18569–18574. doi: 10.1073/pnas.1006980107

Festoff, B. W., Sajja, R. K., van Dreden, P., and Cucullo, L. (2016). HMGB1 and thrombin mediate the blood-brain barrier dysfunction acting as biomarkers of neuroinflammation and progression to neurodegeneration in Alzheimer's disease. *J. Neuroinflammation* 13:194. doi: 10.1186/s12974-016-0670-z

Finnell, J. E., Lombard, C. M., Melson, M. N., Singh, N. P., Nagarkatti, M., Nagarkatti, P., et al. (2017a). The protective effects of resveratrol on social stress-induced cytokine release and depressive-like behavior. *Brain Behav. Immun.* 59, 147–157. doi: 10.1016/j.bbi.2016.08.019

Finnell, J. E., Lombard, C. M., Padi, A. R., Moffitt, C. M., Wilson, L. B., Wood, C. S., et al. (2017b). Physical versus psychological social stress in male rats reveals distinct cardiovascular, inflammatory and behavioral consequences. *PLoS One* 12:e0172868. doi: 10.1371/journal.pone.0172868

Finnell, J. E., Muniz, B. L., Padi, A. R., Lombard, C. M., Moffitt, C. M., Wood, C. S., et al. (2018). Essential role of ovarian hormones in susceptibility to the consequences of witnessing social defeat in female rats. *Biol. Psychiatry* 84, 372–382. doi: 10.1016/j.biopsych.2018.01.013

Finnell, J. E., and Wood, S. K. (2016). Neuroinflammation at the interface of depression and cardiovascular disease: evidence from rodent models of social stress. *Neurobiol. Stress* 4, 1–14. doi: 10.1016/j.ynstr.2016.04.001

Fiske, A., Wetherell, J. L., and Gatz, M. (2009). Depression in older adults. *Annu. Rev. Clin. Psychol.* 5, 363–389. doi: 10.1146/annurev.clinpsy.032408.153621.

Fonken, L. K., Weber, M. D., Daut, R. A., Kitt, M. M., Frank, M. G., Watkins, L. R., et al. (2016). Stress-induced neuroinflammatory priming is time of day dependent. *Psychoneuroendocrinology* 66, 82–90. doi: 10.1016/j.psyneuen.2016.01.006

Frank, M. G., Baratta, M. V., Sprunger, D. B., Watkins, L. R., and Maier, S. F. (2007). Microglia serve as a neuroimmune substrate for stress-induced potentiation of CNS pro-inflammatory cytokine responses. *Brain Behav. Immun.* 21, 47–59. doi: 10.1016/j.bbi.2006.03.005

Frank, M. G., Fonken, L. K., Annis, J. L., Watkins, L. R., and Maier, S. F. (2018). Stress disinhibits microglia via down-regulation of CD200R: a mechanism of neuroinflammatory priming. *Brain Behav. Immun.* 69, 62–73. doi: 10.1016/j.bbi.2017.11.001

Frank, M. G., Thompson, B. M., Watkins, L. R., and Maier, S. F. (2012). Glucocorticoids mediate stress-induced priming of microglial pro-inflammatory responses. *Brain Behav. Immun.* 26, 337–345. doi: 10.1016/j.bbi.2011.10.005

Franklin, T. C., Wohleb, E. S., Zhang, Y., Fogaca, M., Hare, B., and Duman, R. S. (2018). Persistent increase in microglial RAGE contributes to chronic stress-induced priming of depressive-like behavior. *Biol. Psychiatry* 83, 50–60. doi: 10.1016/j.biopsych.2017.06.034

Fruhbeis, C., Frohlich, D., Kuo, W. P., and Kramer-Albers, E. M. (2013). Extracellular vesicles as mediators of neuron-glia communication. *Front. Cell. Neurosci.* 7:182. doi: 10.3389/fncel.2013.00182

Furuta, A., Takashima, S., Yokoo, H., Rothstein, J. D., Wada, K., and Iwaki, T. (2005). Expression of glutamate transporter subtypes during normal human corticogenesis and type II lissencephaly. *Dev. Brain Res.* 155, 155–164. doi: 10.1016/j.devbrainres.2005.01.005

Gallo, J. J., Royall, D. R., and Anthony, J. C. (1993). Risk factors for the onset of depression in middle age and later life. *Soc. Psychiatry Psychiatr. Epidemiol.* 28, 101–108. doi: 10.1007/BF00801739

Gamaro, G. D., Streck, E. L., Matte, C., Prediger, M. E., Wyse, A. T., and Dalmaz, C. (2003). Reduction of hippocampal Na^+, K^+-ATPase activity in rats subjected to an experimental model of depression. *Neurochem. Res.* 28, 1339–1344. doi: 10.1023/A:1024988113978

Ganguly, K., Rejmak, E., Mikosz, M., Nikolaev, E., Knapska, E., and Kaczmarek, L. (2013). Matrix metalloproteinase (MMP) 9 transcription in mouse brain induced by fear learning. *J. Biol. Chem.* 288, 20978–20991. doi: 10.1074/jbc.m113.457903

Gardner, A., Johansson, A., Wibom, R., Nennesmo, I., Von Dobeln, U., Hagenfeldt, L., et al. (2003). Alterations of mitochondrial function and correlations with personality traits in selected major depressive disorder patients. *J. Affect. Disord.* 76, 55–68. doi: 10.1016/s0165-0327(02)00067-8

Gemma, C., and Bachstetter, A. D. (2013). The role of microglia in adult hippocampal neurogenesis. *Front. Cell. Neurosci.* 7:229. doi: 10.3389/fncel.2013.00229

Goel, N., and Bale, T. L. (2009). Examining the intersection of sex and stress in modelling neuropsychiatric disorders. *J. Neuroendocrinol.* 21, 415–420. doi: 10.1111/j.1365-2826.2009.01843.x

Goldwater, D. S., Pavlides, C., Hunter, R. G., Bloss, E. B., Hof, P. R., Mcewen, B. S., et al. (2009). Structural and functional alterations to rat medial prefrontal cortex following chronic restraint stress and recovery. *Neuroscience* 164, 798–808. doi: 10.1016/j.neuroscience.2009.08.053

Gomez, C. R., Boehmer, E. D., and Kovacs, E. J. (2005). The aging innate immune system. *Curr. Opin. Immunol.* 17, 457–462. doi: 10.1016/j.coi.2005.07.013

Gong, Y., Chai, Y., Ding, J. H., Sun, X. L., and Hu, G. (2011). Chronic mild stress damages mitochondrial ultrastructure and function in mouse brain. *Neurosci. Lett.* 488, 76–80. doi: 10.1016/j.neulet.2010.11.006

Gorczynski, R. M. (2005). CD200 and its receptors as targets for immunoregulation. *Curr. Opin. Investig. Drugs* 6, 483–488.

Graham, J. E., Christian, L. M., and Kiecolt-Glaser, J. K. (2006). Stress, age, and immune function: toward a lifespan approach. *J. Behav. Med.* 29, 389–400. doi: 10.1007/s10865-006-9057-4

Gray, P., and Cooney, J. (1982). Stress-induced responses and open-field behavior in estrous and nonestrous mice. *Physiol. Behav.* 29, 287–292. doi: 10.1016/0031-9384(82)90017-8

Greenberg, G. D., Laman-Maharg, A., Campi, K. L., Voigt, H., Orr, V. N., Schaal, L., et al. (2013). Sex differences in stress-induced social withdrawal: role of brain derived neurotrophic factor in the bed nucleus of the stria terminalis. *Front. Behav. Neurosci.* 7:223. doi: 10.3389/fnbeh.2013.00223

Greenberg, G. D., Steinman, M. Q., Doig, I. E., Hao, R., and Trainor, B. C. (2015). Effects of social defeat on dopamine neurons in the ventral tegmental area in male and female California mice. *Eur. J. Neurosci.* 42, 3081–3094. doi: 10.1111/ejn.13099

Haller, J., Fuchs, E., Halasz, J., and Makara, G. B. (1999). Defeat is a major stressor in males while social instability is stressful mainly in females: towards the development of a social stress model in female rats. *Brain Res. Bull.* 50, 33–39. doi: 10.1016/s0361-9230(99)00087-8

Halliwell, B. (1992). Reactive oxygen species and the central nervous system. *J. Neurochem.* 59, 1609–1623. doi: 10.1007/978-3-642-77609-0_2

Hankin, B. L., Abramson, L. Y., Moffitt, T. E., Silva, P. A., Mcgee, R., and Angell, K. E. (1998). Development of depression from preadolescence to young adulthood: emerging gender differences in a 10-year longitudinal study. *J. Abnorm. Psychol.* 107, 128–140. doi: 10.1037/0021-843x.107.1.128

Hansen, I., Klimek, L., Mosges, R., and Hormann, K. (2004). Mediators of inflammation in the early and the late phase of allergic rhinitis. *Curr. Opin. Allergy. Clin. Immunol.* 4, 159–163. doi: 10.1097/00130832-200406000-00004

Harris, A. Z., Atsak, P., Bretton, Z. H., Holt, E. S., Alam, R., Morton, M. P., et al. (2018). A novel method for chronic social defeat stress in female mice. *Neuropsychopharmacology* 43, 1276–1283. doi: 10.1038/npp.2017.259

Harry, G. J., and Kraft, A. D. (2012). Microglia in the developing brain: a potential target with lifetime effects. *Neurotoxicology* 33, 191–206. doi: 10.1016/j.neuro.2012.01.012

Hodes, G. E., Pfau, M. L., Leboeuf, M., Golden, S. A., Christoffel, D. J., Bregman, D., et al. (2014). Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. *Proc. Natl. Acad. Sci. U S A* 111, 16136–16141. doi: 10.1073/pnas.1415191111

Hollis, F., Van Der Kooij, M. A., Zanoletti, O., Lozano, L., Canto, C., and Sandi, C. (2015). Mitochondrial function in the brain links anxiety with social subordination. *Proc. Natl. Acad. Sci. U S A* 112, 15486–15491. doi: 10.1073/pnas.1512653112

Holly, E. N., Shimamoto, A., Debold, J. F., and Miczek, K. A. (2012). Sex differences in behavioral and neural cross-sensitization and escalated cocaine taking as a result of episodic social defeat stress in rats. *Psychopharmacology* 224, 179–188. doi: 10.1007/s00213-012-2846-2

Hubert, G. W., Li, C., Rainnie, D. G., and Muly, E. C. (2014). Effects of stress on AMPA receptor distribution and function in the basolateral amygdala. *Brain Struct. Funct.* 219, 1169–1179. doi: 10.1007/s00429-013-0557-z

Huettner, J. E., and Bean, B. P. (1988). Block of N-methyl-D-aspartate-activated current by the anticonvulsant MK-801: selective binding to open channels. *Proc. Natl. Acad. Sci. U S A* 85, 1307–1311. doi: 10.1073/pnas.85.4.1307

Huffman, J. C., Celano, C. M., Beach, S. R., Motiwala, S. R., and Januzzi, J. L. (2013). Depression and cardiac disease: epidemiology, mechanisms and diagnosis. *Cardiovasc. Psychiatry Neurol.* 2013:695925. doi: 10.1155/2013/695925

Huhman, K. L., Solomon, M. B., Janicki, M., Harmon, A. C., Lin, S. M., Israel, J. E., et al. (2003). Conditioned defeat in male and female Syrian hamsters. *Horm. Behav.* 44, 293–299. doi: 10.1016/j.yhbeh.2003.05.001

Iniguez, S. D., Flores-Ramirez, F. J., Riggs, L. M., Alipio, J. B., Garcia-Carachure, I., Hernandez, M. A., et al. (2018). Vicarious social defeat stress induces depression-related outcomes in female mice. *Biol. Psychiatry* 83, 9–17. doi: 10.1016/j.biopsych.2017.07.014

Irwin, M. R., and Miller, A. H. (2007). Depressive disorders and immunity: 20 years of progress and discovery. *Brain Behav. Immun.* 21, 374–383. doi: 10.1016/j.bbi.2007.01.010

Isaac, J. T. R., Ashby, M. C., and Mcbain, C. J. (2007). The role of the GluR2 subunit in AMPA receptor function and synaptic plasticity. *Neuron* 54, 859–871. doi: 10.1016/j.neuron.2007.06.001

Jacobson-Pick, S., Audet, M.-C., Mcquaid, R. J., Kalvapalle, R., and Anisman, H. (2013). Social agonistic distress in male and female mice: changes of behavior and brain monoamine functioning in relation to acute and chronic challenges. *PLoS One* 8:e60133. doi: 10.1371/journal.pone.0060133

Janeway, C. A. Jr., Travers, P., Walport, M., and Shlomchik, M. J. (2001). *Immunobiology: The Immune System in Health and Disease.* New York, NY: Garland Science.

Jayatissa, M. N., Bisgaard, C., Tingström, A., Papp, M., and Wiborg, O. (2006). Hippocampal cytogenesis correlates to escitalopram-mediated recovery in a chronic mild stress rat model of depression. *Neuropsychopharmacology* 31, 2395–2404. doi: 10.1038/sj.npp.1301041

Jiang, B., Wang, W., Wang, F., Hu, Z.-L., Xiao, J.-L., Yang, S., et al. (2013). The stability of NR2B in the nucleus accumbens controls behavioral and synaptic adaptations to chronic stress. *Biol. Psychiatry* 74, 145–155. doi: 10.1016/j.biopsych.2012.10.031

Joffe, R. T., Levitt, A. J., and Sokolov, S. T. (1996). Augmentation strategies: focus on anxiolytics. *J. Clin. Psychiatry* 57, 25–31; discussion 32–23.

Johnson, F. K., and Kaffman, A. (2018). Early life stress perturbs the function of microglia in the developing rodent brain: new insights and future challenges. *Brain Behav. Immun.* 69, 18–27. doi: 10.1016/j.bbi.2017.06.008

Katon, W. J., Richardson, L., Lozano, P., and Mccauley, E. (2004). The relationship of asthma and anxiety disorders. *Psychosom. Med.* 66, 349–355. doi: 10.1097/00006842-200405000-00010

Kendler, K. S., Kessler, R. C., Heath, A. C., Neale, M. C., and Eaves, L. J. (1991). Coping: a genetic epidemiological investigation. *Psychol. Med.* 21, 337–346. doi: 10.1017/s0033291700020444

Kessler, R. C., McGonagle, K. A., Swartz, M., Blazer, D. G., and Nelson, C. B. (1993). Sex and depression in the National Comorbidity Survey. I: Lifetime prevalence, chronicity and recurrence. *J. Affect. Disord.* 29, 85–96. doi: 10.1016/0165-0327(93)90026-g

Kettenmann, H., Hanisch, U. K., Noda, M., and Verkhratsky, A. (2011). Physiology of microglia. *Physiol. Rev.* 91, 461–553. doi: 10.1152/physrev.00011.2010

Kiecolt-Glaser, J. K., Graham, J. E., Malarkey, W. B., Porter, K., Lemeshow, S., and Glaser, R. (2008). Olfactory influences on mood and autonomic, endocrine and immune function. *Psychoneuroendocrinology* 33, 328–339. doi: 10.1016/j.psyneuen.2007.11.015

Kiecolt-Glaser, J. K., Heffner, K. L., Glaser, R., Malarkey, W. B., Porter, K., Atkinson, C., et al. (2009). How stress and anxiety can alter immediate and late phase skin test responses in allergic rhinitis. *Psychoneuroendocrinology* 34, 670–680. doi: 10.1016/j.psyneuen.2008.11.010

Kiecolt-Glaser, J. K., Preacher, K. J., MacCallum, R. C., Atkinson, C., Malarkey, W. B., and Glaser, R. (2003). Chronic stress and age-related increases in the proinflammatory cytokine IL-6. *Proc. Natl. Acad. Sci. U S A* 100, 9090–9095. doi: 10.1073/pnas.1531903100

Kluver, H., and Bucy, P. C. (1997). Preliminary analysis of functions of the temporal lobes in monkeys. 1939. *J. Neuropsychiatry Clin. Neurosci.* 9, 606–620. doi: 10.1176/jnp.9.4.606

Kobayashi, K., Imagama, S., Ohgomori, T., Hirano, K., Uchimura, K., Sakamoto, K., et al. (2013). Minocycline selectively inhibits M1 polarization of microglia. *Cell Death Dis.* 4:e525. doi: 10.1038/cddis.2013.54

Kohman, R. A., Crowell, B., and Kusnecov, A. W. (2010). Differential sensitivity to endotoxin exposure in young and middle-age mice. *Brain Behav. Immun.* 24, 486–492. doi: 10.1016/j.bbi.2009.12.004

Kollack-Walker, S., Watson, S. J., and Akil, H. (1997). Social stress in hamsters: defeat activates specific neurocircuits within the brain. *J. Neurosci.* 17, 8842–8855. doi: 10.1523/JNEUROSCI.17-22-08842.1997

Koning, N., Swaab, D. F., Hoek, R. M., and Huitinga, I. (2009). Distribution of the immune inhibitory molecules CD200 and CD200R in the normal central nervous system and multiple sclerosis lesions suggests neuron-glia and glia-glia interactions. *J. Neuropathol. Exp. Neurol.* 68, 159–167. doi: 10.1097/nen.0b013e3181964113

Koolhaas, J. M., de Boer, S. F., Buwalda, B., and van Reenen, K. (2007). Individual variation in coping with stress: a multidimensional approach of ultimate and proximate mechanisms. *Brain Behav. Evol.* 70, 218–226. doi: 10.1159/000105485

Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., et al. (1999). Coping styles in animals: current status in behavior and stress-physiology. *Neurosci. Biobehav. Rev.* 23, 925–935. doi: 10.1016/s0149-7634(99)00026-3

Kopp, B. L., Wick, D., and Herman, J. P. (2013). Differential effects of homotypic vs. heterotypic chronic stress regimens on microglial activation in the prefrontal cortex. *Physiol. Behav.* 122, 246–252. doi: 10.1016/j.physbeh.2013.05.030

Kreisel, T., Frank, M. G., Licht, T., Reshef, R., Ben-Menachem-Zidon, O., Baratta, M. V., et al. (2014). Dynamic microglial alterations underlie stress-induced depressive-like behavior and suppressed neurogenesis. *Mol. Psychiatry* 19, 699–709. doi: 10.1038/mp.2013.155

Kumar, S., Hultman, R., Hughes, D., Michel, N., Katz, B. M., and Dzirasa, K. (2014). Prefrontal cortex reactivity underlies trait vulnerability to chronic social defeat stress. *Nat. Commun.* 5:4537. doi: 10.1038/ncomms5537

Lai, S., Wu, G., and Jiang, Z. (2018). Glycyrrhizin treatment facilitates extinction of conditioned fear responses after a single prolonged stress exposure in rats. *Cell. Physiol. Biochem.* 45, 2529–2539. doi: 10.1159/000488271

LaPlant, Q., Chakravarty, S., Vialou, V., Mukherjee, S., Koo, J. W., Kalahasti, G., et al. (2009). Role of nuclear factor κB in ovarian hormone-mediated stress hypersensitivity in female mice. *Biol. Psychiatry* 65, 874–880. doi: 10.1016/j.biopsych.2009.01.024

Lenz, K. M., and Nelson, L. H. (2018). Microglia and beyond: innate immune cells as regulators of brain development and behavioral function. *Front. Immunol.* 9:698. doi: 10.3389/fimmu.2018.00698

Levita, L., Hoskin, R., and Champi, S. (2012). Avoidance of harm and anxiety: a role for the nucleus accumbens. *Neuroimage* 62, 189–198. doi: 10.1016/j.neuroimage.2012.04.059

Li, Y., Howell, E. A., Lagoo, A. S., Kuchibhatla, M., Pan, H., Cohen, H. J., et al. (2009). Differential gene expression of interleukin-1 receptor associated kinase-1 and interleukin-1 receptor associated kinase-M in peripheral blood mononuclear cells of young and aged rats following preconditioning with endotoxin. *Shock* 31, 55–63. doi: 10.1097/shk.0b013e3181778ab2

Li, L.-H., Wang, Z.-C., Yu, J., and Zhang, Y.-Q. (2014). Ovariectomy results in variable changes in nociception, mood and depression in adult female rats. *PLoS One* 9:e94312. doi: 10.1371/journal.pone.0094312

Lindqvist, D., Dhabhar, F. S., James, S. J., Hough, C. M., Jain, F. A., Bersani, F. S., et al. (2017). Oxidative stress, inflammation and treatment response in major depression. *Psychoneuroendocrinology* 76, 197–205. doi: 10.1016/j.psyneuen.2016.11.031

Liu, L. Y., Coe, C. L., Swenson, C. A., Kelly, E. A., Kita, H., and Busse, W. W. (2002). School examinations enhance airway inflammation to antigen challenge. *Am. J. Respir. Crit. Care Med.* 165, 1062–1067. doi: 10.1164/ajrccm.165.8.2109065

Liu, H.-S., Shi, H.-L., Huang, F., Peterson, K. E., Wu, H., Lan, Y.-Y., et al. (2016). Astragaloside IV inhibits microglia activation via glucocorticoid receptor mediated signaling pathway. *Sci. Rep.* 6:19137. doi: 10.1038/srep19137

Loftis, J. M., Huckans, M., and Morasco, B. J. (2010). Neuroimmune mechanisms of cytokine-induced depression: current theories and novel treatment strategies. *Neurobiol. Dis.* 37, 519–533. doi: 10.1016/j.nbd.2009.11.015

Loram, L. C., Sholar, P. W., Taylor, F. R., Wiesler, J. L., Babb, J. A., Strand, K. A., et al. (2012). Sex and estradiol influence glial pro-inflammatory responses to lipopolysaccharide in rats. *Psychoneuroendocrinology* 37, 1688–1699. doi: 10.1016/j.psyneuen.2012.02.018

Lord, J. M., Butcher, S., Killampali, V., Lascelles, D., and Salmon, M. (2001). Neutrophil ageing and immunesenescence. *Mech. Ageing Dev.* 122, 1521–1535. doi: 10.1016/s0047-6374(01)00285-8

Lubach, G. R., Coe, C. L., and Ershler, W. B. (1995). Effects of early rearing environment on immune responses of infant rhesus monkeys. *Brain Behav. Immun.* 9, 31–46. doi: 10.1006/brbi.1995.1004

Lupien, S. J., Maheu, F., Tu, M., Fiocco, A., and Schramek, T. E. (2007). The effects of stress and stress hormones on human cognition: implications for the field of brain and cognition. *Brain Cogn.* 65, 209–237. doi: 10.1016/j.bandc.2007.02.007

MacDonald, J. F., Miljkovic, Z., and Pennefather, P. (1987). Use-dependent block of excitatory amino acid currents in cultured neurons by ketamine. *J. Neurophysiol.* 58, 251–266. doi: 10.1152/jn.1987.58.2.251

Madrigal, J. L., Olivenza, R., Moro, M. A., Lizasoain, I., Lorenzo, P., Rodrigo, J., et al. (2001). Glutathione depletion, lipid peroxidation and mitochondrial dysfunction are induced by chronic stress in rat brain. *Neuropsychopharmacology* 24, 420–429. doi: 10.1016/s0893-133x(00)00208-6

Maier, S. F., and Watkins, L. R. (2005). Stressor controllability and learned helplessness: the roles of the dorsal raphe nucleus, serotonin and corticotropin-releasing factor. *Neurosci. Biobehav. Rev.* 29, 829–841. doi: 10.1016/j.neubiorev.2005.03.021

Marrie, R. A., Walld, R., Bolton, J. M., Sareen, J., Walker, J. R., Patten, S. B., et al. (2017). Increased incidence of psychiatric disorders in immune-mediated inflammatory disease. *J. Psychosom. Res.* 101, 17–23. doi: 10.1016/j.jpsychores.2017.07.015

Martinez, M., Phillips, P. J., and Herbert, J. (1998). Adaptation in patterns of *c-fos* expression in the brain associated with exposure to either single or repeated social stress in male rats. *Eur. J. Neurosci.* 10, 20–33. doi: 10.1046/j.1460-9568.1998.00011.x

Massaad, C. A., and Klann, E. (2011). Reactive oxygen species in the regulation of synaptic plasticity and memory. *Antioxid. Redox Signal.* 14, 2013–2054. doi: 10.1089/ars.2010.3208

Matsui, T., Sekiguchi, M., Hashimoto, A., Tomita, U., Nishikawa, T., and Wada, K. (1995). Functional comparison of D-serine and glycine in rodents: the effect on cloned NMDA receptors and the extracellular concentration. *J. Neurochem.* 65, 454–458. doi: 10.1046/j.1471-4159.1995.65010454.x

Mattson, M. P., Gleichmann, M., and Cheng, A. (2008). Mitochondria in neuroplasticity and neurological disorders. *Neuron* 60, 748–766. doi: 10.1016/j.neuron.2008.10.010

McEwen, B. S., and Gianaros, P. J. (2010). Central role of the brain in stress and adaptation: links to socioeconomic status, health and disease. *Ann. N Y Acad. Sci.* 1186, 190–222. doi: 10.1111/j.1749-6632.2009.05331.x

McKim, D. B., Weber, M. D., Niraula, A., Sawicki, C. M., Liu, X., Jarrett, B. L., et al. (2018). Microglial recruitment of IL-1β-producing monocytes to brain endothelium causes stress-induced anxiety. *Mol. Psychiatry* 23, 1421–1431. doi: 10.1038/mp.2017.64

Menard, C., Pfau, M. L., Hodes, G. E., Kana, V., Wang, V. X., Bouchard, S., et al. (2017). Social stress induces neurovascular pathology promoting depression. *Nat. Neurosci.* 20, 1752–1760. doi: 10.1038/s41593-017-0010-3

Michaluk, P., Wawrzyniak, M., Alot, P., Szczot, M., Wyrembek, P., Mercik, K., et al. (2011). Influence of matrix metalloproteinase MMP-9 on dendritic spine morphology. *J. Cell Sci.* 124, 3369–3380. doi: 10.1242/jcs.090852

Miczek, K. A. (1979). A new test for aggression in rats without aversive stimulation: differential effects of d-amphetamine and cocaine. *Psychopharmacology* 60, 253–259. doi: 10.1007/bf00426664

Miguel-Hidalgo, J. J., Waltzer, R., Whittom, A. A., Austin, M. C., Rajkowska, G., and Stockmeier, C. A. (2010). Glial and glutamatergic markers in depression, alcoholism, and their comorbidity. *J. Affect. Disord.* 127, 230–240. doi: 10.1016/j.jad.2010.06.003

Miller, A. H., Haroon, E., Raison, C. L., and Felger, J. C. (2013). Cytokine targets in the brain: impact on neurotransmitters and neurocircuits. *Depress. Anxiety* 30, 297–306. doi: 10.1002/da.22084

Misaki, M., Suzuki, H., Savitz, J., Drevets, W. C., and Bodurka, J. (2016). Individual variations in nucleus accumbens responses associated with major depressive disorder symptoms. *Sci. Rep.* 6:21227. doi: 10.1038/srep21227

Mitra, R., Sundlass, K., Parker, K. J., Schatzberg, A. F., and Lyons, D. M. (2006). Social stress-related behavior affects hippocampal cell proliferation in mice. *Physiol. Behav.* 89, 123–127. doi: 10.1016/j.physbeh.2006.05.047

Moieni, M., Irwin, M. R., Jevtic, I., Olmstead, R., Breen, E. C., and Eisenberger, N. I. (2015). Sex differences in depressive and socioemotional responses to an inflammatory challenge: implications for sex differences in depression. *Neuropsychopharmacology* 40, 1709–1716. doi: 10.1038/npp.2015.17

Mujica-Parodi, L. R., Cha, J., and Gao, J. (2017). From anxious to reckless: a control systems approach unifies prefrontal-limbic regulation across the spectrum of threat detection. *Front. Syst. Neurosci.* 11:18. doi: 10.3389/fnsys.2017.00018

Muramatsu, R., Takahashi, C., Miyake, S., Fujimura, H., Mochizuki, H., and Yamashita, T. (2012). Angiogenesis induced by CNS inflammation promotes neuronal remodeling through vessel-derived prostacyclin. *Nat. Med.* 18, 1658–1664. doi: 10.1038/nm.2943

Murrough, J. W., Huryk, K. M., Mao, X., Iacoviello, B., Collins, K., Nierenberg, A. A., et al. (2018). A pilot study of minocycline for the treatment of bipolar depression: effects on cortical glutathione and oxidative stress *in vivo*. *J. Affect. Disord.* 230, 56–64. doi: 10.1016/j.jad.2017.12.067

Nair, A., and Bonneau, R. H. (2006). Stress-induced elevation of glucocorticoids increases microglia proliferation through NMDA receptor activation. *J. Neuroimmunol.* 171, 72–85. doi: 10.1016/j.jneuroim.2005.09.012

Nasca, C., Zelli, D., Bigio, B., Piccinin, S., Scaccianoce, S., Nisticò, R., et al. (2015). Stress dynamically regulates behavior and glutamatergic gene expression in hippocampus by opening a window of epigenetic plasticity. *Proc. Natl. Acad. Sci. U S A* 112, 14960–14965. doi: 10.1073/pnas.1516016112

Nelson, R. J., and Trainor, B. C. (2007). Neural mechanisms of aggression. *Nat. Rev. Neurosci.* 8, 536–546. doi: 10.1038/nrn2174

Neylan, T. C., Sun, B., Rempel, H., Ross, J., Lenoci, M., O'Donovan, A., et al. (2011). Suppressed monocyte gene expression profile in men versus women with PTSD. *Brain Behav. Immun.* 25, 524–531. doi: 10.1016/j.bbi.2010.12.001

Nicolai, K. A., Laney, T., and Mezulis, A. H. (2013). Different stressors, different strategies, different outcomes: how domain-specific stress responses differentially predict depressive symptoms among adolescents. *J. Youth Adolesc.* 42, 1183–1193. doi: 10.1007/s10964-012-9866-4

Nimmerjahn, A., Kirchhoff, F., and Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science* 308, 1314–1318. doi: 10.1126/science.1110647

Nolen-Hoeksema, S. (2001). Gender differences in depression. *Curr. Dir. Psychol. Sci.* 10, 173–176. doi: 10.1111/1467-8721.00142

Oliveira, L. A., Almeida, J., Benini, R., and Crestani, C. C. (2015). CRF1 and CRF2 receptors in the bed nucleus of the stria terminalis modulate the

cardiovascular responses to acute restraint stress in rats. *Pharmacol. Res.* 95–96, 53–62. doi: 10.1016/j.phrs.2015.03.012

Ota, K. T., Liu, R.-J., Voleti, B., Maldonado-Aviles, J. G., Duric, V., Iwata, M., et al. (2014). REDD1 is essential for stress-induced synaptic loss and depressive behavior. *Nat. Med.* 20, 531–535. doi: 10.1038/nm.3513

Pace, T. W., Mletzko, T. C., Alagbe, O., Musselman, D. L., Nemeroff, C. B., Miller, A. H., et al. (2006). Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. *Am. J. Psychiatry* 163, 1630–1633. doi: 10.1176/appi.ajp.163.9.1630

Pandey, S. P., Rai, R., Gaur, P., and Prasad, S. (2015). Development- and age-related alterations in the expression of AMPA receptor subunit GluR2 and its trafficking proteins in the hippocampus of male mouse brain. *Biogerontology* 16, 317–328. doi: 10.1007/s10522-014-9548-6

Patki, G., Solanki, N., Atrooz, F., Allam, F., and Salim, S. (2013). Depression, anxiety-like behavior and memory impairment are associated with increased oxidative stress and inflammation in a rat model of social stress. *Brain Res.* 1539, 73–86. doi: 10.1016/j.brainres.2013.09.033

Pearson-Leary, J., Eacret, D., Chen, R., Takano, H., Nicholas, B., and Bhatnagar, S. (2017). Inflammation and vascular remodeling in the ventral hippocampus contributes to vulnerability to stress. *Transl. Psychiatry* 7:e1160. doi: 10.1038/tp.2017.122

Qiao, H., Li, M. X., Xu, C., Chen, H. B., An, S. C., and Ma, X. M. (2016). Dendritic spines in depression: what we learned from animal models. *Neural Plast.* 2016:8056370. doi: 10.1155/2016/8056370

Qiu, J., Xu, J., Zheng, Y., Wei, Y., Zhu, X., Lo, E. H., et al. (2010). High-mobility group box 1 promotes metalloproteinase-9 upregulation through Toll-like receptor 4 after cerebral ischemia. *Stroke* 41, 2077–2082. doi: 10.1161/strokeaha.110.590463

Quinn, A. M., Williams, A. R., Sivilli, T. I., Raison, C. L., and Pace, T. W. W. (2018). The plasma interleukin-6 response to acute psychosocial stress in humans is detected by a magnetic multiplex assay: comparison to high-sensitivity ELISA. *Stress* 21, 376–381. doi: 10.1080/10253890.2018.1446518

Radley, J. J., and Morrison, J. H. (2005). Repeated stress and structural plasticity in the brain. *Ageing Res. Rev.* 4, 271–287. doi: 10.1016/j.arr.2005.03.004

Radley, J. J., Rocher, A. B., Rodriguez, A., Ehlenberger, D. B., Dammann, M., McEwen, B. S., et al. (2008). Repeated stress alters dendritic spine morphology in the rat medial prefrontal cortex. *J. Comp. Neurol.* 507, 1141–1150. doi: 10.1002/cne.21588

Rajkowska, G., Miguel-Hidalgo, J. J., Dubey, P., Stockmeier, C. A., and Krishnan, K. R. R. (2005). Prominent reduction in pyramidal neurons density in the orbitofrontal cortex of elderly depressed patients. *Biol. Psychiatry* 58, 297–306. doi: 10.1016/j.biopsych.2005.04.013

Rajkowska, G., and Stockmeier, C. A. (2013). Astrocyte pathology in major depressive disorder: insights from human postmortem brain tissue. *Curr. Drug. Targets* 14, 1225–1236. doi: 10.2174/13894501113149990156

Reddy, M. S. (2010). Depression: the disorder and the burden. *Indian J. Psychol. Med.* 32, 1–2. doi: 10.4103/0253-7176.70510

Renault, P. F., Hoofnagle, J. H., Park, Y., Mullen, K. D., Peters, M., Jones, D. B., et al. (1987). Psychiatric complications of long-term interferon alfa therapy. *Arch. Intern. Med.* 147, 1577–1580. doi: 10.1001/archinte.147.9.1577

Rezin, G. T., Cardoso, M. R., Gonçalves, C. L., Scaini, G., Fraga, D. B., Riegel, R. E., et al. (2008). Inhibition of mitochondrial respiratory chain in brain of rats subjected to an experimental model of depression. *Neurochem. Int.* 53, 395–400. doi: 10.1016/j.neuint.2008.09.012

Riba, M., Wulsin, L., and Rubenfire, M. (2011). *Psychiatry and Heart Disease: The Mind, Brain, and Heart.* Hoboken, NJ: Wiley-Blackwell.

Robinson, K. E., Pearson, M. M., Cannistraci, C. J., Anderson, A. W., Kuttesch, J. F. Jr., Wymer, K., et al. (2015). Functional neuroimaging of working memory in survivors of childhood brain tumors and healthy children: associations with coping and psychosocial outcomes. *Child Neuropsychol.* 21, 779–802. doi: 10.1080/09297049.2014.924492

Rochfort, K. D., Collins, L. E., Murphy, R. P., and Cummins, P. M. (2014). Downregulation of blood-brain barrier phenotype by proinflammatory cytokines involves NADPH oxidase-dependent ROS generation: consequences for interendothelial adherens and tight junctions. *PLoS One* 9:e101815. doi: 10.1371/journal.pone.0101815

Roque, S., Mesquita, A. R., Palha, J. A., Sousa, N., and Correia-Neves, M. (2014). The behavioral and immunological impact of maternal separation: a matter of timing. *Front. Behav. Neurosci.* 8:192. doi: 10.3389/fnbeh.2014.00192

Ros-Bernal, F., Hunot, S., Herrero, M. T., Parnadeau, S., Corvol, J.-C., Lu, L., et al. (2011). Microglial glucocorticoid receptors play a pivotal role in regulating dopaminergic neurodegeneration in parkinsonism. *Proc. Natl. Acad. Sci. U S A* 108, 6632–6637. doi: 10.1073/pnas.1017820108

Salim, S., Asghar, M., Chugh, G., Taneja, M., Xia, Z., and Saha, K. (2010). Oxidative stress: a potential recipe for anxiety, hypertension and insulin resistance. *Brain Res.* 1359, 178–185. doi: 10.1016/j.brainres.2010.08.093

Salim, S., Asghar, M., Taneja, M., Hovatta, I., Chugh, G., Vollert, C., et al. (2011). Potential contribution of oxidative stress and inflammation to anxiety and hypertension. *Brain Res.* 1404, 63–71. doi: 10.1016/j.brainres.2011.06.024

Sandberg, S., Paton, J. Y., Ahola, S., McCann, D. C., McGuinness, D., Hillary, C. R., et al. (2000). The role of acute and chronic stress in asthma attacks in children. *Lancet* 356, 982–987. doi: 10.1016/s0140-6736(00)02715-x

Sarkar, S., Malovic, E., Harishchandra, D. S., Ghaisas, S., Panicker, N., Charli, A., et al. (2017). Mitochondrial impairment in microglia amplifies NLRP3 inflammasome proinflammatory signaling in cell culture and animal models of Parkinson's disease. *NPJ Parkinsons Dis.* 3:30. doi: 10.1038/s41531-017-0032-2

Sasayama, D., Hattori, K., Wakabayashi, C., Teraishi, T., Hori, H., Ota, M., et al. (2013). Increased cerebrospinal fluid interleukin-6 levels in patients with schizophrenia and those with major depressive disorder. *J. Psychiatr. Res.* 47, 401–406. doi: 10.1016/j.jpsychires.2012.12.001

Satpute, A. B., and Lieberman, M. D. (2006). Integrating automatic and controlled processes into neurocognitive models of social cognition. *Brain Res.* 1079, 86–97. doi: 10.1016/j.brainres.2006.01.005

Schoenfeld, T. J., McCausland, H. C., Morris, H. D., Padmanaban, V., and Cameron, H. A. (2017). Stress and loss of adult neurogenesis differentially reduce hippocampal volume. *Biol. Psychiatry* 82, 914–923. doi: 10.1016/j.biopsych.2017.05.013

Schwarz, J. M., Sholar, P. W., and Bilbo, S. D. (2012). Sex differences in microglial colonization of the developing rat brain. *J. Neurochem.* 120, 948–963. doi: 10.1111/j.1471-4159.2011.07630.x

Segerstrom, S. C., and Miller, G. E. (2004). Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. *Psychol. Bull.* 130, 601–630. doi: 10.1037/0033-2909.130.4.601

Seo, J. H., Miyamoto, N., Hayakawa, K., Pham, L. D., Maki, T., Ayata, C., et al. (2013). Oligodendrocyte precursors induce early blood-brain barrier opening after white matter injury. *J. Clin. Invest.* 123, 782–786. doi: 10.1172/JCI65863

Setiawan, E., Wilson, A. A., Mizrahi, R., Rusjan, P. M., Miler, L., Rajkowska, G., et al. (2015). Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes. *JAMA Psychiatry* 72, 268–275. doi: 10.1001/jamapsychiatry.2014.2427

Sheline, Y. I., Barch, D. M., Donnelly, J. M., Ollinger, J. M., Snyder, A. Z., and Mintun, M. A. (2001). Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. *Biol. Psychiatry* 50, 651–658. doi: 10.1016/s0006-3223(01)01263-x

Shimamoto, A., Debold, J. F., Holly, E. N., and Miczek, K. A. (2011). Blunted accumbal dopamine response to cocaine following chronic social stress in female rats: exploring a link between depression and drug abuse. *Psychopharmacology* 218, 271–279. doi: 10.1007/s00213-011-2364-7

Shirayama, Y., and Chaki, S. (2006). Neurochemistry of the nucleus accumbens and its relevance to depression and antidepressant action in rodents. *Curr. Neuropharmacol.* 4, 277–291. doi: 10.2174/157015906778520773

Sih, A., Bell, A. M., Johnson, J. C., and Ziemba, R. E. (2004). Behavioral syndromes: an intergrative overiew. *Q. Rev. Biol.* 79, 241–277. doi: 10.1086/422893

Silveira Villarroel, H., Bompolaki, M., Mackay, J. P., Miranda Tapia, A. P., Michaelson, S. D., Leitermann, R. J., et al. (2018). NPY induces stress resilience via downregulation of I_h in principal neurons of rat basolateral amygdala. *J. Neurosci.* 38, 4505–4520. doi: 10.1523/JNEUROSCI.3528-17.2018

Simon, M., Czéh, B., and Fuchs, E. (2005). Age-dependent susceptibility of adult hippocampal cell proliferation to chronic psychosocial stress. *Brain Res.* 1049, 244–248. doi: 10.1016/j.brainres.2005.05.006

Slavich, G. M., Way, B. M., Eisenberger, N. I., and Taylor, S. E. (2010). Neural sensitivity to social rejection is associated with inflammatory responses to social stress. *Proc. Natl. Acad. Sci. U S A* 107, 14817–14822. doi: 10.1073/pnas.1009164107

Solanki, N., Salvi, A., Patki, G., and Salim, S. (2017). Modulating oxidative stress relieves stress-induced behavioral and cognitive impairments in rats. *Int. J. Neuropsychopharmacol.* 20, 550–561. doi: 10.1093/ijnp/pyx017

Stauder, A., and Kovács, M. (2003). Anxiety symptoms in allergic patients: identification and risk factors. *Psychosom. Med.* 65, 816–823. doi: 10.1097/01.psy.0000088620.66211.b1

Stelzhammer, V., Ozcan, S., Gottschalk, M. G., Steeb, H., Hodes, G. E., Guest, P. C., et al. (2015). Central and peripheral changes underlying susceptibility and resistance to social defeat stress—a proteomic profiling study. *Diagn. Neuropsychiatry* 1, 1–7. doi: 10.1016/j.dineu.2015.08.001

Straube, T., Mentzel, H. J., and Miltner, W. H. (2007). Waiting for spiders: brain activation during anticipatory anxiety in spider phobics. *Neuroimage* 37, 1427–1436. doi: 10.1016/j.neuroimage.2007.06.023

Strawbridge, R., Arnone, D., Danese, A., Papadopoulos, A., Herane Vives, A., and Cleare, A. J. (2015). Inflammation and clinical response to treatment in depression: a meta-analysis. *Eur. Neuropsychopharmacol.* 25, 1532–1543. doi: 10.1016/j.euroneuro.2015.06.007

Takahashi, A., Chung, J. R., Zhang, S., Zhang, H., Grossman, Y., Aleyasin, H., et al. (2017). Establishment of a repeated social defeat stress model in female mice. *Sci. Rep.* 7:12838. doi: 10.1038/s41598-017-12811-8

Takaki, J., Fujimori, K., Miura, M., Suzuki, T., Sekino, Y., and Sato, K. (2012). L-glutamate released from activated microglia downregulates astrocytic L-glutamate transporter expression in neuroinflammation: the 'collusion' hypothesis for increased extracellular L-glutamate concentration in neuroinflammation. *J. Neuroinflammation* 9:275. doi: 10.1186/1742-2094-9-275

Takeuchi, H., Jin, S., Wang, J., Zhang, G., Kawanokuchi, J., Kuno, R., et al. (2006). Tumor necrosis factor-α induces neurotoxicity via glutamate release from hemichannels of activated microglia in an autocrine manner. *J. Biol. Chem.* 281, 21362–21368. doi: 10.1074/jbc.M600504200

Tang, J., Yu, W., Chen, S., Gao, Z., and Xiao, B. (2018). Microglia polarization and endoplasmic reticulum stress in chronic social defeat stress induced depression mouse. *Neurochem. Res.* 43, 985–994. doi: 10.1007/s11064-018-2504-0

Trainor, B. C., Pride, M. C., Villalon Landeros, R., Knoblauch, N. W., Takahashi, E. Y., Silva, A. L., et al. (2011). Sex differences in social interaction behavior following social defeat stress in the monogamous California mouse (*Peromyscus californicus*). *PLoS One* 6:e17405. doi: 10.1371/journal.pone.0017405

Trivedi, M. H., Rush, A. J., Wisniewski, S. R., Nierenberg, A. A., Warden, D., Ritz, L., et al. (2006). Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *Am. J. Psychiatry* 163, 28–40. doi: 10.1176/appi.ajp.163.1.28

Tse, Y. C., Montoya, I., Wong, A. S., Mathieu, A., Lissemore, J., Lagace, D. C., et al. (2014). A longitudinal study of stress-induced hippocampal volume changes in mice that are susceptible or resilient to chronic social defeat. *J. Neurosci.* 24, 1120–1128. doi: 10.1002/hipo.22296

Tynan, R. J., Naicker, S., Hinwood, M., Nalivaiko, E., Buller, K. M., Pow, D. V., et al. (2010). Chronic stress alters the density and morphology of microglia in a subset of stress-responsive brain regions. *Brain Behav. Immun.* 24, 1058–1068. doi: 10.1016/j.bbi.2010.02.001

Vaváková, M., Ďuračková, Z., and Trebatická, J. (2015). Markers of oxidative stress and neuroprogression in depression disorder. *Oxid. Med. Cell. Longev.* 2015:898393. doi: 10.1155/2015/898393

Ver Hoeve, E. S., Kelly, G., Luz, S., Ghanshani, S., and Bhatnagar, S. (2013). Short-term and long-term effects of repeated social defeat during adolescence or adulthood in female rats. *Front. Behav. Neurosci.* 249, 63–73. doi: 10.1016/j.neuroscience.2013.01.073

Videbech, P. (2000). PET measurements of brain glucose metabolism and blood flow in major depressive disorder: a critical review. *Acta. Psychiatr. Scand.* 101, 11–20. doi: 10.1034/j.1600-0447.2000.101001011.x

Villa, A., Vegeto, E., Poletti, A., and Maggi, A. (2016). Estrogens, neuroinflammation, and neurodegeneration. *Endocr. Rev.* 37, 372–402. doi: 10.1210/er.2016-1007

Voorhees, J. L., Tarr, A. J., Wohleb, E. S., Godbout, J. P., Mo, X., Sheridan, J. F., et al. (2013). Prolonged restraint stress increases IL-6, reduces IL-10 and causes persistent depressive-like behavior that is reversed by recombinant IL-10. *PLoS One* 8:e58488. doi: 10.1371/journal.pone.0058488

Wang, X. B., Bozdagi, O., Nikitczuk, J. S., Zhai, Z. W., Zhou, Q., and Huntley, G. W. (2008). Extracellular proteolysis by matrix metalloproteinase-9 drives dendritic spine enlargement and long-term potentiation coordinately. *Proc. Natl. Acad. Sci. U S A* 105, 19520–19525. doi: 10.1073/pnas.0807248105

Wang, Y. L., Han, Q. Q., Gong, W. Q., Pan, D. H., Wang, L. Z., Hu, W., et al. (2018). Microglial activation mediates chronic mild stress-induced depressive- and anxiety-like behavior in adult rats. *J. Neuroinflammation* 15:21. doi: 10.1186/s12974-018-1054-3

Wang, K., Ye, L., Lu, H., Chen, H., Zhang, Y., Huang, Y., et al. (2017). TNF-α promotes extracellular vesicle release in mouse astrocytes through glutaminase. *J. Neuroinflammation* 14:87. doi: 10.1186/s12974-017-0853-2

Wang, J., Zhang, K., Chen, X., Liu, X., Teng, H., Zhao, M., et al. (2017). Epigenetic activation of ASCT2 in the hippocampus contributes to depression-like behavior by regulating D-serine in mice. *Front. Mol. Neurosci.* 10:139. doi: 10.3389/fnmol.2017.00139

Warren, B. L., Vialou, V. F., Iniguez, S. D., Alcantara, L. F., Wright, K. N., Feng, J., et al. (2013). Neurobiological sequelae of witnessing stressful events in adult mice. *Biol. Psychiatry* 73, 7–14. doi: 10.1016/j.biopsych.2012.06.006

Watanabe, Y., Gould, E., Cameron, H. A., Daniels, D. C., and Mcewen, B. S. (1992). Phenytoin prevents stress- and corticosterone-induced atrophy of CA3 pyramidal neurons. *Hippocampus* 2, 431–435. doi: 10.1002/hipo.450020410

Weber, M. D., Frank, M. G., Tracey, K. J., Watkins, L. R., and Maier, S. F. (2015). Stress induces the danger-associated molecular pattern HMGB-1 in the hippocampus of male Sprague Dawley rats: a priming stimulus of microglia and the NLRP3 inflammasome. *J. Neurosci.* 35, 316–324. doi: 10.1523/jneurosci.3561-14.2015

Weinberger, A. H., Gbedemah, M., Martinez, A. M., Nash, D., Galea, S., and Goodwin, R. D. (2017). Trends in depression prevalence in the USA from 2005 to 2015: widening disparities in vulnerable groups. *Psychol. Med.* 48, 1308–1315. doi: 10.1017/s0033291717002781

Weinhard, L., Di Bartolomei, G., Bolasco, G., Machado, P., Schieber, N. L., Neniskyte, U., et al. (2018). Microglia remodel synapses by presynaptic trogocytosis and spine head filopodia induction. *Nat. Commun.* 9:1228. doi: 10.1038/s41467-018-03566-5

Weissman, M. M., and Klerman, G. L. (1992). Depression: current understanding and changing trends. *Annu. Rev. Public Health* 13, 319–339. doi: 10.1146/annurev.pu.13.050192.001535

Wickens, M. M., Bangasser, D. A., and Briand, L. A. (2018). Sex differences in psychiatric disease: a focus on the glutamate system. *Front. Mol. Neurosci.* 11:197. doi: 10.3389/fnmol.2018.00197

Wilson, M. A., Grillo, C. A., Fadel, J. R., and Reagan, L. P. (2015). Stress as a one-armed bandit: differential effects of stress paradigms on the morphology, neurochemistry and behavior in the rodent amygdala. *Neurobiol. Stress* 1, 195–208. doi: 10.1016/j.ynstr.2015.06.001

Wohleb, E. S., Mckim, D. B., Sheridan, J. F., and Godbout, J. P. (2014a). Monocyte trafficking to the brain with stress and inflammation: a novel axis of immune-to-brain communication that influences mood and behavior. *Front. Neurosci.* 8:447. doi: 10.3389/fnins.2014.00447

Wohleb, E. S., Patterson, J. M., Sharma, V., Quan, N., Godbout, J. P., and Sheridan, J. F. (2014b). Knockdown of interleukin-1 receptor type-1 on endothelial cells attenuated stress-induced neuroinflammation and prevented anxiety-like behavior. *J. Neurosci.* 34, 2583–2591. doi: 10.1523/jneurosci.3723-13.2014

Wohleb, E. S., Powell, N. D., Godbout, J. P., and Sheridan, J. F. (2013). Stress-induced recruitment of bone marrow-derived monocytes to the brain promotes anxiety-like behavior. *J. Neurosci.* 33, 13820–13833. doi: 10.1523/jneurosci.1671-13.2013

Wood, S. K. (2014). Individual differences in the neurobiology of social stress: implications for depression-cardiovascular disease comorbidity. *Curr. Neuropharmacol.* 12, 205–211. doi: 10.2174/1570159x11666131120224413

Wood, C. S., Valentino, R. J., and Wood, S. K. (2017). Individual differences in the locus coeruleus-norepinephrine system: relevance to stress-induced cardiovascular vulnerability. *Physiol. Behav.* 101, 40–48. doi: 10.1016/j.physbeh.2016.07.008

Wood, S. K., Walker, H. E., Valentino, R. J., and Bhatnagar, S. (2010). Individual differences in reactivity to social stress predict susceptibility and resilience to a

depressive phenotype: role of corticotropin-releasing factor. *Endocrinology* 151, 1795–1805. doi: 10.1210/en.2009-1026

Wood, S. K., Wood, C. S., Lombard, C. M., Lee, C. S., Zhang, X. Y., Finnell, J. E., et al. (2015). Inflammatory factors mediate vulnerability to a social stress-induced depressive-like phenotype in passive coping rats. *Biol. Psychiatry* 78, 38–48. doi: 10.1016/j.biopsych.2014.10.026

Wood, G. E., Young, L. T., Reagan, L. P., Chen, B., and Mcewen, B. S. (2004). Stress-induced structural remodeling in hippocampus: prevention by lithium treatment. *Proc. Natl. Acad. Sci. U S A* 101, 3973–3978. doi: 10.1073/pnas.0400208101

Wood, S. K., Zhang, X. Y., Reyes, B. A., Lee, C. S., Van Bockstaele, E. J., and Valentino, R. J. (2013). Cellular adaptations of dorsal raphe serotonin neurons associated with the development of active coping in response to social stress. *Biol. Psychiatry* 73, 1087–1094. doi: 10.1016/j.biopsych.2013.01.026

Wu, S.-Z., Bodles, A. M., Porter, M. M., Griffin, W. S. T., Basile, A. S., and Barger, S. W. (2004). Induction of serine racemase expression and D-serine release from microglia by amyloid β-peptide. *J. Neuroinflammation* 1:2. doi: 10.1186/1742-2094-1-2

Wu, X., Liu, B. J., Ji, S., Wu, J. F., Xu, C. Q., Du, Y. J., et al. (2015). Social defeat stress promotes tumor growth and angiogenesis by upregulating vascular endothelial growth factor/extracellular signal-regulated kinase/matrix metalloproteinase signaling in a mouse model of lung carcinoma. *Mol. Med. Rep.* 12, 1405–1412. doi: 10.3892/mmr.2015.3559

Yadid, G., Overstreet, D. H., and Zangen, A. (2001). Limbic dopaminergic adaptation to a stressful stimulus in a rat model of depression. *Brain Res.* 896, 43–47. doi: 10.1016/s0006-8993(00)03248-0

Yan, W., Chang, Y., Liang, X., Cardinal, J. S., Huang, H., Thorne, S. H., et al. (2012). High-mobility group box 1 activates caspase-1 and promotes hepatocellular carcinoma invasiveness and metastases. *Hepatology* 55, 1863–1875. doi: 10.1002/hep.25572

Yang, H., Lundback, P., Ottosson, L., Erlandsson-Harris, H., Venereau, E., Bianchi, M. E., et al. (2012). Redox modification of cysteine residues regulates the cytokine activity of high mobility group box-1 (HMGB1). *Mol. Med.* 18, 250–259. doi: 10.2119/molmed.2011.00389

Yassa, M. A., Hazlett, R. L., Stark, C. E., and Hoehn-Saric, R. (2012). Functional MRI of the amygdala and bed nucleus of the stria terminalis during conditions of uncertainty in generalized anxiety disorder. *J. Psychiatr. Res.* 46, 1045–1052. doi: 10.1016/j.jpsychires.2012.04.013

Ye, L., Huang, Y., Zhao, L., Li, Y., Sun, L., Zhou, Y., et al. (2013). IL-1β and TNF-α induce neurotoxicity through glutamate production: a potential role for neuronal glutaminase. *J. Neurochem.* 125, 897–908. doi: 10.1111/jnc.12263

Zarate, C. A. Jr., Singh, J. B., Carlson, P. J., Brutsche, N. E., Ameli, R., Luckenbaugh, D. A., et al. (2006). A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch. Gen. Psychiatry* 63, 856–864. doi: 10.1001/archpsyc.63.8.856

Zhang, J., Takahashi, H. K., Liu, K., Wake, H., Liu, R., Maruo, T., et al. (2011). Anti-high mobility group box-1 monoclonal antibody protects the blood-brain barrier from ischemia-induced disruption in rats. *Stroke* 42, 1420–1428. doi: 10.1161/strokeaha.110.598334

Zhao, L., Huang, Y., Tian, C., Taylor, L., Curthoys, N., Wang, Y., et al. (2012). Interferon-α regulates glutaminase 1 promoter through STAT1 phosphorylation: relevance to HIV-1 associated neurocognitive disorders. *PLoS One* 7:e32995. doi: 10.1371/journal.pone.0032995

Zhu, G., Okada, M., Yoshida, S., Mori, F., Ueno, S., Wakabayashi, K., et al. (2006). Effects of interleukin-1β on hippocampal glutamate and GABA releases associated with Ca^{2+}-induced Ca^{2+} releasing systems. *Epilepsy Res.* 71, 107–116. doi: 10.1016/j.eplepsyres.2006.05.017

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Behavioral Neuroscience

BRIEF RESEARCH REPORT
published: 29 August 2018
doi: 10.3389/fnbeh.2018.00181

Paternal Care Impacts Oxytocin Expression in California Mouse Offspring and Basal Testosterone in Female, but Not Male Pups

Christine N. Yohn[1,2], Amanda B. Leithead[1], Julian Ford[1], Alexander Gill[1] and Elizabeth A. Becker[1]*

[1]*Department of Psychology, Saint Joseph's University, Philadelphia, PA, United States,* [2]*Department of Psychology, Rutgers University, Piscataway, NJ, United States*

Natural variations in parenting are associated with differences in expression of several hormones and neuropeptides which may mediate lasting effects on offspring development, like regulation of stress reactivity and social behavior. Using the bi-parental California mouse, we have demonstrated that parenting and aggression are programmed, at least in part, by paternal behavior as adult offspring model the degree of parental behavior received in development and are more territorial following high as compared to low levels of care. Development of these behaviors may be driven by transient increases in testosterone following paternal retrievals and increased adult arginine vasopressin (AVP) immunoreactivity within the bed nucleus of the stria terminalis (BNST) among high-care (HC) offspring. It remains unclear, however, whether other neuropeptides, such as oxytocin (OT), which is sensitive to gonadal steroids, are similarly impacted by father-offspring interactions. To test this question, we manipulated paternal care (high and low care) and examined differences in adult offspring OT-immunoreactive (OT-ir) within social brain areas as well as basal T and corticosterone (Cort) levels. HC offspring had more OT-ir within the paraventricular nucleus (PVN) and supraoptic nucleus (SON) than low-care (LC) offspring. Additionally, T levels were higher among HC than LC females, but no differences were found in males. There were no differences in Cort indicating that our brief father-pup separations likely had no consequences on stress reactivity. Together with our previous work, our data suggest that social behavior may be programmed by paternal care through lasting influences on the neuroendocrine system.

Keywords: oxytocin, testosterone, corticosterone, paternal care, *Peromyscus californicus*

OPEN ACCESS

Edited by:
Erica R. Glasper, University of Maryland, College Park, United States

Reviewed by:
Brian Trainor, University of California, Davis, United States
Karen Lisa Bales, University of California, Davis, United States

****Correspondence:***
Christine N. Yohn
cy253@scarletmail.rutgers.edu

Received: *31 May 2018*
Accepted: *02 August 2018*
Published: *29 August 2018*

Citation:
Yohn CN, Leithead AB, Ford J, Gill A and Becker EA (2018) Paternal Care Impacts Oxytocin Expression in California Mouse Offspring and Basal Testosterone in Female, but Not Male Pups. Front. Behav. Neurosci. 12:178. doi: 10.3389/fnbeh.2018.00181

Abbreviations: AVP, arginine vasopressin; BNST, bed nucleus of the stria terminalis; CORT, corticosterone; HC, highcare; HGL, huddling, licking and grooming behavior; HPA, hypothalamic-pituitary-adrenal; -ir, immunoreactive; LC, lowcare; NGS, normal goat serum; OT, oxytocin; PBS, phosphate buffer saline; PVN, paraventricular nucleus; SON, supraoptic nucleus; T, testosterone.

INTRODUCTION

Variability within postnatal environments can have profound consequences on phenotype development in offspring. Stress reactivity and parental behavior, among other things, are programmed by the quality of care received (Bester-Meredith and Marler, 2003; Shannon et al., 2005; Ichise et al., 2006; Uriarte et al., 2007; Rosenfeld et al., 2013). This phenotypic plasticity is accompanied by changes to neural pathways associated with behavioral regulation (Champagne et al., 2004; Weaver et al., 2006; Oomen et al., 2009). While mothers are the primary caregiver in most mammalian species, in an estimated 5%–10% of mammals, fathers also contribute to offspring development (Gubernick and Alberts, 1987; Ziegler et al., 2000; Bester-Meredith and Marler, 2001). Within the bi-parental California mouse (*Peromyscus californicus*), fathers provide high-care (HC) towards both same and opposite sex offspring, which influence neuroendocrine mechanisms that facilitate similar rather than sexually dimorphic development. Transient increases in testosterone levels (Becker et al., 2010; Chary et al., 2015) and greater arginine vasopressin (AVP) expression in the bed nucleus of the stria terminalis (BNST; Frazier et al., 2006; Yohn et al., 2017), accompany territorial aggression in HC offspring. Whereas in most species parental behavior is accompanied by a reduction in aggression and T, in bi-parental species (Hume and Wynne-Edwards, 2005), like the territorial California mouse (Trainor and Marler, 2001, 2002), T remains high in fathers and is important for maintaining paternal behavior since castration reduces paternal behavior in this species (Trainor and Marler, 2001). The current study aimed to identify whether paternal care, which we have demonstrated programs both territoriality (Yohn et al., 2017) and parental behavior (Bester-Meredith and Marler, 2003; Gleason and Marler, 2013; Becker, unpublished; Leithead, unpublished) in adult California mouse offspring, influences other neuroendocrine mechanisms in addition to T and AVP that may act or interact to shape adult behavior.

The neuropeptide oxytocin (OT) is a likely candidate since it is synthesized in the paraventricular nucleus (PVN) and supraoptic nucleus (SON), with projections to social brain areas (Champagne et al., 2001 *rats*; Pedersen and Boccia, 2003 *rats*) that regulate social behaviors (Consiglio et al., 2005 *rats*) including parenting (Bales et al., 2007 *prairie voles*; Neumann and van den Burg, 2013 *rats*) and aggression, particularly parental aggression (Bosch, 2013 "*rodents*") and hypothalamus-pituitary-adrenal (HPA) function (Neumann et al., 2000 *rats*; Engelmann et al., 2006 *rats*; Rault et al., 2013 *pigs*). Moreover, the OT system is sensitive to gonadal steroids, such as T (reviews see Pedersen, 1997; Sladek et al., 2000 *rat*; Gordon et al., 2011), which alone or by aromatization into estradiol acts as a modulator of OT secretion and receptor expression within brain areas (i.e., hypothalamus) that regulate both reproductive and parenting behavior (Johnson et al., 1991 *rats*; Okabe et al., 2013 *mice*; Gordon et al., 2017 *humans*). Furthermore, there is significant overlap in expression of OT and aromatase enzymes within the mammalian brain, with aromatase also mainly expressed within the hypothalamus and limbic system (Naftolin et al., 2001; Trainor et al., 2006; El-Emam Dief et al., 2013). Furthermore, developmental studies, in mandarin voles, indicate paternally deprived offspring have lower OT receptor expression than offspring raised with a father (Wang et al., 2012; Cao et al., 2014). Whether this is due to the absence of the caregiver or a particular behavior displayed by the father is unknown.

In addition to changes in the brain, environmental influences on social behaviors and stress reactivity may be mediated by alterations to endocrine systems (Bale, 2006; Clinton et al., 2008; Lajud et al., 2012; Carini and Nephew, 2013). For example, California mouse offspring experience transient increases in T in response to paternal retrievals (Becker et al., 2010; Chary et al., 2015). It is possible that experiencing these brief increases in T will result in overall increased basal T levels in adulthood since postnatal T is correlated with adult T (Sachser and Proöve, 1988; Lürzel et al., 2010), although this hypothesis has yet to be tested. Additionally, paternal deprivation leads to deficits in social behavior (Yu et al., 2012; Bambico et al., 2015) and increased anxiety (McEwen, 2007; Roberts et al., 2007; Jia et al., 2009; Kim et al., 2013), which may correlate with HPA dysregulation since in response to stress, corticosterone (Cort) is secreted. However, no transient differences in Cort in response to paternal care (Becker et al., 2010; Chary et al., 2015) nor basal Cort dissimilarities between paternal absence or presence (Wang et al., 2012) have been reported.

Postnatal paternal care impacts the development of social behaviors and may be regulated by a complex interplay between the hormones OT, T and Cort. For instance, OT expression can be increased via T (El-Emam Dief et al., 2013), and then have a buffering effect on the HPA axis, leading to a decrease in Cort release (Leuner et al., 2012). To examine long-term effects of postnatal paternal interactions on neuroendocrine system development, we manipulated California mouse offspring rearing conditions to receive either HC or low-care (LC). Given that removal of the father leads to decreased OT expression (Wang et al., 2012; Cao et al., 2014), we aimed to assess whether variability in paternal care leads to plasticity within the OT system. In the current study, our primary aim was to assess paternal care impact on OT-immunoreactive (OT-ir) cell distribution in the PVN and SON, with these three areas of the brain sensitive to gonadal steroids (El-Emam Dief et al., 2013) and important in regulating stress response (Dabrowska et al., 2011). Therefore, we also assessed adult basal T and Cort levels since the paternal care manipulation can also have long-term effects on the endocrine system. We hypothesized that PVN and SON OT-ir would be higher in HC than LC offspring. Since California mouse pups experience transient increases in T following paternal retrievals (Becker et al., 2010; Chary et al., 2015) and postnatal surges in T lead to higher adult T levels (Sachser and Proöve, 1988; Lürzel et al., 2010), we predicted higher basal T levels in adult HC than LC offspring. Lastly, as a manipulation check, we measured basal Cort levels to confirm that our modified retrieval manipulation had no lasting effects on stress reactivity; predicting similar basal Cort levels between HC and LC offspring.

MATERIALS AND METHODS

This study was carried out in accordance with the recommendations of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Saint Joseph's University IACUC. Detailed materials and methods are provided as **Supplementary Material** Information.

Subjects

Brains were collected from 52 California mice adults (120 days) from the same cohort of mice used in Yohn et al. (2017) study. Briefly, experimental animals postnatal days (PND) 15–21 were assigned randomly to either HC (n = 26) or LC (n = 26) postnatal paternal rearing conditions. As previously described by Yohn et al. (2017), we used a modified retrieval manipulation (use of Plexiglas divider) to ensure populations of experimental animals had distinct early life experiences (HC and LC). To establish populations, experimental families were first moved into an observational cage on PND 8. Subsequently, paternal retrieval behavior was manipulated for 20 min daily, across PND 15–21 when pups are more inclined to leave the nest and paternal retrieval behavior is at its highest (Bester-Meredith et al., 1999). During each daily manipulation the mother and non-experimental pups were removed and housed separately. The experimental pup was handled for 30 s and returned to the cage either inside the nest (LC) or outside of the nest (HC). Prior to the pups return to the cage, a Plexiglass divider was inserted into the cage separating the large and small compartments of the observational cage. Results from Yohn et al. (2017) showed that across 7 days, HC experienced greater paternal retrievals than LC offspring since the Plexiglass divider (had plastic mesh barrier for LC group) inhibited father-pup contact during the daily LC manipulations. Separation of father and pup had no effect on paternal behavior in the LC group once the father and pup were reunited (Yohn et al., 2017).

Immunohistochemistry

Adult experimental mice taken from the colony room were euthanized via rapid decapitation. Brains were extracted, immediately fixed in 5% acrolein overnight at 4°C, transferred to a 20% sucrose buffer solution and refrigerated for 48 h, and finally frozen on dry ice and stored at −80°C until cutting. Beginning from approximately Bregma 0.37 through −1.23 (Paxinos et al., 2007; Campi et al., 2013), brains were sliced on a cryostat at 40 um and stored at −80°C in cryoprotectant until staining. Sections were incubated overnight in a previously validated antibody (Trainor et al., 2010) rabbit anti-OT (1:1,000, AB911, Millipore, Temecula, CA, USA) and then for 2 h in goat anti-rabbit IgG (1:250, PI-1,000, Vector Labs, Burlingame, CA, USA) both times diluted in 2% normal goat serum (NGS) phosphate buffer saline (PBS). Next, sections were amplified in Avidin Biotin Complex (Vector Labs, Burlingame, CA, USA) and then visualized using DAB peroxidase substrate kit (Vector Labs, Burlingame, CA, USA). PBS washes occurred before and after incubation, amplification and visualization.

Image Analysis

Sections were photographed on a Leica DM 2000 outfitted with a DFC310 FX digital color camera (Leica) at 10× magnification. For all cell counts, number of OT-ir positive cells were averaged across two images of the PVN (posterior, Bregma −0.70 thru −94) and SON (Bregma −0.70 thru −94; **Figure 1A**).

Testosterone and Corticosterone Enzyme Immunoassay

Trunk blood was collected at brain extraction, with enough serum from 39 of 52 experimental mice (male = 20, female = 19). After collection, samples were centrifuged and separated, then stored at −80°C until assayed. Plasma T (1:10 dilution) and Cort (1:50 dilution) concentrations were determined using commercial assay kits (Enzo Life Sciences, Farmingdale, NY, USA) previously validated in the California mouse (Chary et al., 2015). The standard curve slope generated for Cort had a slope of 1 (r^2 = 0.91) and the slope for T was 0.72 (r^2 = 0.87). Inter-assay coefficient of variability values were 1.1% (Cort) and 3.1% (T) with intra-assay coefficient of variability values being 1.87% (Cort) and 2.65% (T). The cross-reactivity of the Cort kits, according to the manufacturer, was 100% for Cort, 28.6% for deoxycorticosterone, 1.7% for progesterone, and negligible for other steroid hormones (>1%). The cross-reactivity of the T kits was 100% for T, 14.64% for 19-hydroxytestosterone, 7.20% for androstendione, and negligible for other steroid hormones (>1%). Kit sensitivity was 26.99 pg/mL for Cort and 5.67 pg/mL for T.

Statistical Analyses

Separate 2 × 2 analysis of variance (ANOVA) were run to assess early life rearing conditions and sex differences on PVN and SON OT-ir and basal T and CORT levels. *Post hoc* independent samples t-tests for within-sex differences were run. Pearson's correlations were run to assess relationship between paternal behavior and expression of OT-ir within each area. One animal (female LC) was removed from analyses as cell counts were only obtained from one of the three regions and T levels were three standard deviations above the mean. All statistical analyses were conducted using SPSS (version 23.0, Chicago, IL, USA).

RESULTS

OT-Immunoreactivity

Separate ANOVAs indicated HC offspring had significantly more OT-ir in the PVN ($F_{(1,48)}$ = 17.49, p < 0.001; **Figure 1B**) and SON ($F_{(1,39)}$ = 9.1, p = 0.004; **Figure 1C**) than LC offspring. Planned *post hoc* comparisons revealed HC males had significantly more OT-ir than LC males within the PVN ($t_{(25)}$ = 2.89, p = 0.008; **Figure 1B**) and SON ($t_{(22)}$ = 3.6, p = 0.002; **Figure 1C**). Within females, HC offspring had more OT-ir in the PVN ($t_{(23)}$ = 3.01, p = 0.006; **Figure 1B**) than LC offspring. Unlike males, SON OT was similarly expressed between HC and LC females (p = 0.421; **Figure 1C**). We observed no effect of sex nor an interaction between

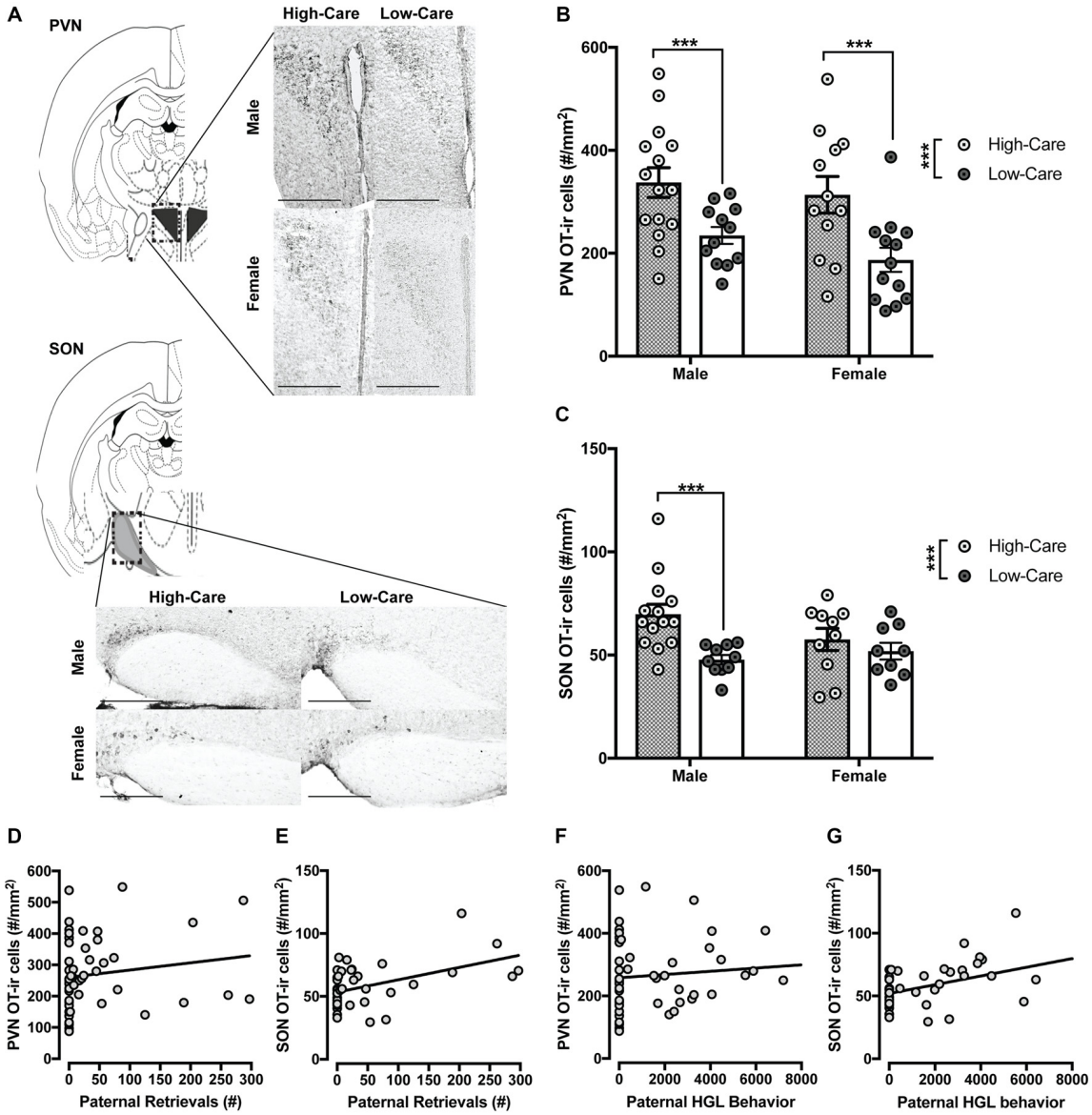

FIGURE 1 | Differences in distribution of oxytocin-immunoreactive (OT-ir) cells between high-care (HC) and low-care (LC) offspring. Representative images of OT-ir staining for each area of interest (atlas images were reproduced from Paxinos mouse atlas, 2007; 10× magnification with scale bar = 500 μm) **(A)**. HC male and female offspring have higher OT-ir cells within the paraventricular nucleus (PVN; **B**) and supraoptic nucleus (SON; **C**) compared to LC offspring. Number of postnatal paternal is significantly associated with amount of OT-ir cells within the PVN **(D)** and SON **(E)**. Amount of postnatal paternal huddling, grooming and licking (HGL) behavior also was significantly associated with distribution of OT-ir cells in these areas **(F,G)**. ***p-value < 0.001, *p-value < 0.05.

rearing condition and sex on OT-ir within these regions (p's > 0.2).

Relationship Between OT-Immunoreactivity and Early Life Rearing Conditions

Paternal retrievals were positively correlated with PVN ($r = 0.44$, $p = 0.02$; **Figure 1D**) and SON ($r = 0.47$, $p < 0.001$; **Figure 1E**) OT-ir. Additionally, paternal huddling, grooming and licking (HGL) behavior was positively correlated with PVN ($r = 0.34$, $p = 0.016$; **Figure 1F**), SON ($r = 0.44$, $p = 0.003$; **Figure 1G**) OT-ir.

Plasma Corticosterone and Testosterone Concentrations

As expected, males had higher plasma T levels than females ($F_{(1,33)} = 7.58$, $p = 0.009$; **Figure 2A**). While there was no main effect of rearing condition on plasma T concentrations ($p = 0.7$) nor differences among males ($p = 0.69$); rearing effects were indicated with higher T levels in HC than LC females,

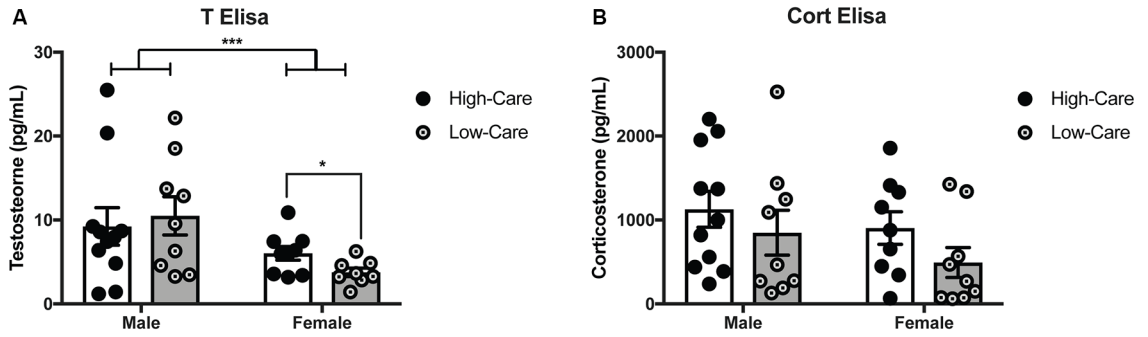

FIGURE 2 | Differences in early life paternal care on hormonal levels. HC females have higher T levels than LC females, with males having higher overall testosterone levels females **(A)**. Corticosterone (Cort) levels did not differ between HC and LC adult offspring across both sexes **(B)**. ***p-value < 0.001, *p-value < 0.05.

($t_{(15)} = 2.27$, $p = 0.039$). No rearing effects nor an interaction effect on plasma T levels (p's > 0.05) were found.

No rearing effects, sex differences, nor an interaction were found for plasma Cort concentrations (p's > 0.05; **Figure 2B**).

DISCUSSION

Variability in paternal care produced differences in offspring OT-ir. Pups raised under HC conditions had greater OT-ir than LC offspring within the PVN and SON, regions that regulate various behaviors ranging from parenting (Neumann and van den Burg, 2013; Wang et al., 2015) to affective behaviors and autonomic functions (Cao et al., 2014; Yee et al., 2016). Within the PVN, OT expression is linked to the onset and maintenance of both maternal (Neumann and van den Burg, 2013) and paternal care (Kenkel et al., 2014). Since paternal care programs adult offspring social behavior in the California mouse (Bester-Meredith and Marler, 2001; Frazier et al., 2006; Yohn et al., 2017), we suggest that these differences in OT may guide the development of these distinct behavioral phenotypes. Previous studies delineate the relationship between paternal care and OT receptor expression within the PVN and SON (Wang et al., 2012; Cao et al., 2014), our novel findings illustrate susceptibility of PVN, and SON OT-ir to postnatal paternal care. However, unlike paternal deprivation studies, we emphasize the importance of paternal behaviors on adult OT-ir with high levels of both paternal retrievals and HGL behavior positively related to PVN and SON OT-ir. In response to paternal retrievals HC offspring experience transient increases in T (Becker et al., 2010; Chary et al., 2015), with OT expression within these brain areas regulated in part by T (Sladek et al., 2000; Gordon et al., 2011) and the aromatization of T into estradiol (Naftolin et al., 2001; Trainor et al., 2006; El-Emam Dief et al., 2013). The PVN and SON contain high levels of aromatase (El-Emam Dief et al., 2013), which could further explain differences in PVN and SON OT-ir between HC and LC offspring. In the California mouse male and female offspring retrieve their offspring at similar levels as they received during development (Bester-Meredith and Marler, 2003; Becker, unpublished; Leithead, unpublished), however mechanisms for this behavioral transmission are not fully elucidated. Since OT receptor expression is related to level of postnatal care (Francis et al., 2002; Perkeybile et al., 2015), our results suggest a potential mechanism by which parental care is transmitted across generations.

Since sex differences in OT are reported (Lee et al., 2009; Carter, 2014), we tested for paternal effects on OT-ir within each sex even though no overall sex differences were observed. Our analyses revealed HC males and females had more OT-ir within the PVN than LC offspring, suggesting OT-ir is plastic in response to the environment, potentially allowing more adapted social behaviors within HC offspring. However, within the SON, only males were susceptible to variability in care, which may be due to sex differences in social behaviors and physiological functions that SON OT-ir regulates. In females and males SON OT is associated with parenting and other social behaviors (Song et al., 2010; Bales et al., 2011); with SON OT also facilitating uterine contraction and lactation in females (Higashida et al., 2013). While future maternal care may be susceptible to postnatal rearing conditions, other functions like uterine contractions and lactation may be resistant to environmental fluctuations in OT, thus resulting in HC and LC females having similar expression within the SON. Alternatively, this null result could have been confounded by estrous, since SON OT-ir fluctuates in relation to circulating estrogen levels (Shughrue et al., 2002) and we did not track estrous cycle.

Since paternal retrievals induce transient increases in T (Becker et al., 2010; Chary et al., 2015) and postnatal surges in T, may be related to adult T levels (Sachser and Proöve, 1988; Lürzel et al., 2010), we wanted to assess long-term effects of rearing condition on adult basal T levels. Not surprisingly, we found males had higher basal T levels than females. Within females, we observed greater T levels in HC than LC offspring, which is likely a long-term effect of rearing condition. Consistent with Wang et al. (2015) prairie vole study, we found no differences in male T. It is possible that postnatal paternal interactions may not have long term effects on male T, or since males already have high T, that a ceiling effect (Evans et al., 2000) may obscure a potential impact. Seeing as OT expression is associated with a buffering effect on HPA function (Neumann et al., 2000; Leuner et al., 2012) and T can have organizational effects on the HPA axis

(Seale et al., 2005; Goel and Bale, 2008), we examined adult basal Cort levels as a manipulation check since parental interactions may influence HPA function (Slotten et al., 2006; Engert et al., 2011). Consistent with paternal deprivation studies and our previous work (Becker et al., 2010; Wang et al., 2012; Chary et al., 2015; Yohn et al., 2017) basal Cort levels were similar between HC and LC offspring, suggesting our manipulation had no lasting effects on Cort. To further test the impact of our manipulation on stress responsivity, future studies could measure Cort levels in adult mice after experiencing a stressful situation.

Our results demonstrate developmental plasticity within the OT system in response to the postnatal paternal environment which may be mediated by transient changes in T subsequent to paternal retrievals during development. Our study is the first to illustrate long-term effects of paternal care on basal T levels in females, which may mediate transmission of social behaviors, like parenting and aggression in territorial species. Future studies are needed to examine the relationship between postnatal increases in T in response to paternal retrievals and adult OT expression to delineate whether the interplay between postnatal T and adult OT mediate changes in social behavior. Our results emphasize the critical role fathers hold in the development of the neuroendocrine system in both males and females.

AUTHOR CONTRIBUTIONS

CY designed, conducted the behavioral manipulations, collected both the tissue and plasma used in this study. Additionally, CY ran all the statistical analyses and wrote the manuscript. AL assisted in behavioral manipulations, tissue collection and in conducting the Elisa's. She also helped in the manuscript preparation by giving thoughtful feedback/input on each draft. JF contributed to this project through conducting the immunohistochemistry and imaging all stained sections. AG assisted with this project by scoring behavioral videos collected during each of the 7 days of experimental manipulation. He also assisted with the Elisa's. EB was the principal investigator of this project and gave guidance/assistance on all phases of this project. Additionally, she assisted in the manuscript preparation.

FUNDING

This research was supported by a Psi Chi Grant to EB and a Sigma Xi Grant to CY.

ACKNOWLEDGMENTS

We thank Joey Collins, Kieran Slattery, Jamie Palmer, Seattle Heiman and Linda Muller for their assistance.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnbeh.2018.00181/full#supplementary-material

REFERENCES

Bale, T. L. (2006). Stress sensitivity and the development of affective disorders. *Horm. Behav.* 50, 529–533. doi: 10.1016/j.yhbeh.2006.06.033

Bales, K. L., Boone, E., Epperson, P., Hoffman, G., and Carter, C. S. (2011). Are behavioral effects of early experience mediated by oxytocin? *Front. Psychiatry* 2:24. doi: 10.3389/fpsyt.2011.00024

Bales, K. L., van Westerhuyzen, J. A., Lewis-Reese, A. D., Grotte, N. D., Lanter, J. A., and Carter, C. S. (2007). Oxytocin has dose-dependent developmental effects on pair-bonding and alloparental care in female prairie voles. *Horm. Behav.* 52, 274–279. doi: 10.1016/j.yhbeh.2007.05.004

Bambico, F. R., Lacoste, B., Hattan, P. R., and Gobbi, G. (2015). Father absence in the monogamous California mouse impairs social behavior and modifies dopamine and glutamate synapses in the medial prefrontal cortex. *Cereb. Cortex* 25, 1163–1175. doi: 10.1093/cercor/bht310

Becker, E. A., Moore, B. M., Auger, C., and Marler, C. A. (2010). Paternal behavior increases testosterone levels in offspring of the California mouse. *Horm. Behav.* 58, 385–389. doi: 10.1016/j.yhbeh.2010.03.019

Bester-Meredith, J. K., and Marler, C. A. (2001). Vasopressin and aggression in cross-fostered California mice (*Peromyscus californicus*) and white-footed mice (*Peromyscus leucopus*). *Horm. Behav.* 40, 51–64. doi: 10.1006/hbeh.2001.1666

Bester-Meredith, J. K., and Marler, C. A. (2003). Vasopressin and the transmission of paternal behavior across generations in mated, cross-fostered *Peromyscus* mice. *Behav. Neurosci.* 117, 455–463. doi: 10.1037/0735-7044.117.3.455

Bester-Meredith, J. K., Young, L. J., and Marler, C. A. (1999). Species differences in paternal behavior and aggression in *Peromyscus* and their associations with vasopressin immunoreactivity and receptors. *Horm. Behav.* 36, 25–38. doi: 10.1006/hbeh.1999.1522

Bosch, O. J. (2013). Maternal aggression in rodents: brain oxytocin and vasopressin mediate pup defence. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 368:20130085. doi: 10.1098/rstb.2013.0085

Campi, K. L., Jameson, C. E., and Trainor, B. C. (2013). Sexual dimorphism in the brain of the monogamous California mouse (Peromyscus californicus). *Brain behav. evol.* 81, 236–249. doi: 10.1159/000353260

Cao, Y., Wu, R., Tai, F., Zhang, X., Yu, P., An, X., et al. (2014). Neonatal paternal deprivation impairs social recognition and alters levels of oxytocin and estrogen receptor α mRNA expression in the MeA and NAcc and serum oxytocin in mandarin voles. *Horm. Behav.* 65, 57–65. doi: 10.1016/j.yhbeh.2013.11.005

Carini, L. M., and Nephew, B. C. (2013). Effects of early life social stress on endocrinology, maternal behavior and lactation in rats. *Horm. Behav.* 64, 634–641. doi: 10.1016/j.yhbeh.2013.08.011

Carter, C. S. (2014). Oxytocin pathways and the evolution of human behavior. *Annu. Rev. Psychol.* 65, 17–39. doi: 10.1146/annurev-psych-010213-115110

Champagne, F. A., Chretien, P., Stevenson, C. W., Zhang, T. Y., Gratton, A., and Meaney, M. J. (2004). Variations in nucleus accumbens dopamine associated with individual differences in maternal behavior in the rat. *J. Neurosci.* 24, 4113–4123. doi: 10.1523/JNEUROSCI.5322-03.2004

Champagne, F. A., Diorio, J., Sharma, S., and Meaney, M. J. (2001). Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. *Proc. Natl. Acad. Sci. U S A* 98, 12736–12741. doi: 10.1073/pnas.221224598

Chary, M. C., Cruz, J. P., Bardi, M., and Becker, E. A. (2015). Paternal retrievals increase testosterone levels in both male and female California mouse (*Peromyscus californicus*) offspring. *Horm. Behav.* 73, 23–29. doi: 10.1016/j.yhbeh.2015.05.013

Clinton, S., Miller, S., Watson, S. J., and Akil, H. (2008). Prenatal stress does not alter innate novelty-seeking behavioral traits, but differentially affects individual differences in neuroendocrine stress responsivity. *Psychoneuroendocrinology* 33, 162–177. doi: 10.1016/j.psyneuen.2007.10.012

Consiglio, A. R., Borsoi, A., Pereira, G. A., and Lucion, A. B. (2005). Effects of oxytocin microinjected into the central amygdaloid nucleus and bed nucleus

of stria terminalis on maternal aggressive behavior in rats. *Physiol. Behav.* 85, 354–362. doi: 10.1016/j.physbeh.2005.05.002

Dabrowska, J., Hazra, R., Ahern, T. H., Guo, J. D., McDonald, A. J., Mascagni, F., et al. (2011). Neuroanatomical evidence for reciprocal regulation of the corticotrophin-releasing factor and oxytocin systems in the hypothalamus and the bed nucleus of the stria terminalis of the rat: implications for balancing stress and affect. *Psychoneuroendocrinology* 36, 1312–1326. doi: 10.1016/j.psyneuen.2011.03.003

El-Emam Dief, A., Caldwell, J. D., and Jirikowski, G. F. (2013). Colocalization of P450 aromatase and oxytocin immunostaining in the rat hypothalamus. *Horm. Metab. Res.* 45, 273–276. doi: 10.1055/s-0032-1327680

Engelmann, M., Ebner, K., Landgraf, R., and Wotjak, C. T. (2006). Effects of Morris water maze testing on the neuroendocrine stress response and intrahypothalamic release of vasopressin and oxytocin in the rat. *Horm. Behav.* 50, 496–501. doi: 10.1016/j.yhbeh.2006.04.009

Engert, V., Efanov, S. I., Dedovic, K., Dagher, A., and Pruessner, J. C. (2011). Increased cortisol awakening response and afternoon/evening cortisol output in healthy young adults with low early life parental care. *Psychopharmacology* 214, 261–268. doi: 10.1007/s00213-010-1918-4

Evans, M. R., Goldsmith, A. R., and Norris, S. R. (2000). The effects of testosterone on antibody production and plumage coloration in male house sparrows (*Passer domesticus*). *Behav. Ecol. Sociobiol.* 47, 156–163. doi: 10.1007/s002650050006

Francis, D. D., Young, L. J., Meaney, M. J., and Insel, T. R. (2002). Naturally occurring differences in maternal care are associated with the expression of oxytocin and vasopressin (V1a) receptors: gender differences. *J. Neuroendocrinol.* 14, 349–353. doi: 10.1046/j.0007-1331.2002.00776.x

Frazier, C. R., Trainor, B. C., Cravens, C. J., Whitney, T. K., and Marler, C. A. (2006). Paternal behavior influences development of aggression and vasopressin expression in male California mouse offspring. *Horm. Behav.* 50, 699–707. doi: 10.1016/j.yhbeh.2006.06.035

Gleason, E. D., and Marler, C. A. (2013). Non-genomic transmission of paternal behaviour between fathers and sons in the monogamous and biparental California mouse. *Proc. Biol. Sci.* 280:20130824. doi: 10.1098/rspb.2013.0824

Goel, N., and Bale, T. L. (2008). Organizational and activational effects of testosterone on masculinization of female physiological and behavioral stress responses. *Endocrinology* 149, 6399–6405. doi: 10.1210/en.2008-0433

Gordon, I., Martin, C., Feldman, R., and Leckman, J. F. (2011). Oxytocin and social motivation. *Dev. Cogn. Neurosci.* 1, 471–493. doi: 10.1016/j.dcn.2011.07.007

Gordon, I., Pratt, M., Bergunde, K., Zagoory-Sharon, O., and Feldman, R. (2017). Testosterone, oxytocin, and the development of human parental care. *Horm. Behav.* 93, 184–192. doi: 10.1016/j.yhbeh.2017.05.016

Gubernick, D. J., and Alberts, J. R. (1987). The biparental care system of the California mouse, *Peromyscus californicus*. *J. Comp. Psychol.* 101, 169–177. doi: 10.1037/0735-7036.101.2.169

Higashida, H., Lopatina, O., and Sarwat, A. (2013). "Regulation of oxytocin and vasopressin secretion," in *Oxytocin, Vasopressin, and Related Peptides in the Regulation of Behavior*, eds E. Choleris, D. W. Pfaff and M. Kavaliers (Cambridge: Cambridge University Press), 44–55.

Hume, J. M., and Wynne-Edwards, K. E. (2005). Castration reduces male testosterone, estradiol, and territorial aggression, but not paternal behavior in biparental dwarf hamsters (*Phodopus campbelli*). *Horm. Behav.* 48, 303–310. doi: 10.1016/j.yhbeh.2005.04.001

Ichise, M., Vines, D. C., Gura, T., Anderson, G. M., Suomi, S. J., Higley, J. D., et al. (2006). Effects of early life stress on [^{11}C]DASB positron emission tomography imaging of serotonin transporters in adolescent peer- and mother-reared rhesus monkeys. *J. Neurosci.* 26, 4638–4643. doi: 10.1523/JNEUROSCI.5199-05.2006

Jia, R., Tai, F., An, S., Zhang, X., and Broders, H. (2009). Effects of neonatal paternal deprivation or early deprivation on anxiety and social behaviors of the adults in mandarin voles. *Behav. Processes* 82, 271–278. doi: 10.1016/j.beproc.2009.07.006

Johnson, A. E., Coirini, H., Insel, T. R., and McEwen, B. S. (1991). The regulation of oxytocin receptor binding in the ventromedial hypothalamic nucleus by testosterone and its metabolites. *Endocrinology* 128, 891–896. doi: 10.1210/endo-128-2-891

Kenkel, W. M., Suboc, G., and Carter, C. S. (2014). Autonomic, behavioral and neuroendocrine correlates of paternal behavior in male prairie voles. *Physiol. Behav.* 128, 252–259. doi: 10.1016/j.physbeh.2014.02.006

Kim, J. G., Jung, H. S., Kim, K. J., Min, S. S., and Yoon, B. J. (2013). Basal blood corticosterone level is correlated with susceptibility to chronic restraint stress in mice. *Neurosci. Lett.* 555, 137–142. doi: 10.1016/j.neulet.2013.09.031

Lajud, N., Roque, A., Cajero, M., Gutiérrez-Ospina, G., and Torner, L. (2012). Periodic maternal separation decreases hippocampal neurogenesis without affecting basal corticosterone during the stress hyporesponsive period, but alters HPA axis and coping behavior in adulthood. *Psychoneuroendocrinology* 37, 410–420. doi: 10.1016/j.psyneuen.2011.07.011

Lee, H. J., Macbeth, A. H., Pagani, J. H., and Young, W. S. III. (2009). Oxytocin: the great facilitator of life. *Prog. Neurobiol.* 88, 127–151. doi: 10.1016/j.pneurobio.2009.04.001

Leuner, B., Caponiti, J. M., and Gould, E. (2012). Oxytocin stimulates adult neurogenesis even under conditions of stress and elevated glucocorticoids. *Hippocampus* 22, 861–868. doi: 10.1002/hipo.20947

Lürzel, S., Kaiser, S., and Sachser, N. (2010). Social interaction, testosterone, and stress responsiveness during adolescence. *Physiol. Behav.* 99, 40–46. doi: 10.1016/j.physbeh.2009.10.005

McEwen, B. S. (2007). Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol. Rev.* 87, 873–904. doi: 10.1152/physrev.00041.2006

Naftolin, F., Horvath, T. L., and Balthazart, J. (2001). Estrogen synthetase (aromatase) immunohistochemistry reveals concordance between avian and rodent limbic systems and hypothalami. *Exp. Biol. Med. Maywood* 226, 717–725. doi: 10.1177/153537020222600802

Neumann, I. D., and van den Burg, E. (2013). "Oxytocin and vasopressin release and their receptor-mediated intracellular pathways that determine their behavioral effects," in *Oxytocin, Vasopressin, and Related Peptides in the Regulation of Behavior*, eds E. Choleris, D. Pfaff, and M. Kavaliers (Cambridge: Cambridge University Press), 27–43.

Neumann, I. D., Wigger, A., Torner, L., Holsboer, F., and Landgraf, R. (2000). Brain oxytocin inhibits basal and stress-induced activity of the hypothalamo-pituitary-adrenal axis in male and female rats: partial action within the paraventricular nucleus. *J. Neuroendocrinol.* 12, 235–243. doi: 10.1046/j.1365-2826.2000.00442.x

Okabe, S., Kitano, K., Nagasawa, M., Mogi, K., and Kikusui, T. (2013). Testosterone inhibits facilitating effects of parenting experience on parental behavior and the oxytocin neural system in mice. *Physiol. Behav.* 118, 159–164. doi: 10.1016/j.physbeh.2013.05.017

Oomen, C. A., Girardi, C. E., Cahyadi, R., Verbeek, E. C., Krugers, H., Joels, M., et al. (2009). Opposite effects of early maternal deprivation on neurogenesis in male versus female rats. *PLoS One* 4:e3675. doi: 10.1371/journal.pone.0003675

Paxinos, G., Koutcherov, Y., Halliday, G. M., and Watson, C., Wang, H. (2007). *Atlas of the Developing Mouse Brain: at E17.5, P0, and P6.* Amsterdam: Academic Press.

Pedersen, C. A. (1997). Oxytocin control of maternal behavior. *Ann. N Y Acad. Sci.* 807, 126–145. doi: 10.1111/j.1749-6632.1997.tb51916.x

Pedersen, C. A., and Boccia, M. L. (2003). Oxytocin antagonism alters rat dams' oral grooming and upright posturing over pups. *Physiol. Behav.* 80, 233–241. doi: 10.1016/j.physbeh.2003.07.011

Perkeybile, A. M., Delaney-Busch, N., Hartman, S., Grimm, K. J., and Bales, K. L. (2015). Intergenerational transmission of alloparental behavior and oxytocin and vasopressin receptor distribution in the prairie vole. *Front. Behav. Neurosci.* 9:191. doi: 10.3389/fnbeh.2015.00191

Rault, J. L., Carter, C. S., Garner, J. P., Marchant-Forde, J. N., Richert, B. T., and Lay, D. C. Jr. (2013). Repeated intranasal oxytocin administration in early life dysregulates the HPA axis and alters social behavior. *Physiol. Behav.* 112–113, 40–48. doi: 10.1016/j.physbeh.2013.02.007

Roberts, M. L., Buchanan, K. L., Hasselquist, D., and Evans, M. R. (2007). Effects of testosterone and corticosterone on immunocompetence in the zebra finch. *Horm. Behav.* 51, 126–134. doi: 10.1016/j.yhbeh.2006.09.004

Rosenfeld, C. S., Johnson, S. A., Ellersieck, M. R., and Roberts, R. M. (2013). Interactions between parents and parents and pups in the monogamous California mouse (*Peromyscus californicus*). *PLoS One* 8:e75725. doi: 10.1371/journal.pone.0075725

Sachser, N., and Proöve, E. (1988). Plasma-testosterone development in colony and individually housed male guinea pigs. *Ethology* 79, 62–70. doi: 10.1111/j.1439-0310.1988.tb00699.x

Seale, J. V., Wood, S. A., Atkinson, H. C., Lightman, S. L., and Harbuz, M. S. (2005). Organizational role for testosterone and estrogen on adult hypothalamic-pituitary-adrenal axis activity in the male rat. *Endocrinology* 146, 1973–1982. doi: 10.1210/en.2004-1201

Shannon, C., Schwandt, M. L., Champoux, M., Shoaf, S. E., Suomi, S. J., Linnoila, M., et al. (2005). Maternal absence and stability of individual differences in CSF 5-HIAA concentrations in rhesus monkey infants. *Am. J. Psychiatry* 162, 1658–1664. doi: 10.1176/appi.ajp.162.9.1658

Shughrue, P. J., Dellovade, T. L., and Merchenthaler, I. (2002). Estrogen modulates oxytocin gene expression in regions of the rat supraoptic and paraventricular nuclei that contain estrogen receptor-β. *Prog. Brain Res.* 139, 15–29. doi: 10.1016/s0079-6123(02)39004-6

Sladek, C. D., Swenson, K. L., Kapoor, R., and Sidorowicz, H. E. (2000). The role of steroid hormones in the regulation of vasopressin and oxytocin release and mRNA expression in hypothalamo-neurohypophysial explants from the rat. *Exp. Physiol.* 85, 171S–177S. doi: 10.1111/j.1469-445x.2000.tb00021.x

Slotten, H. A., Kalinichev, M., Hagan, J. J., Marsden, C. A., and Fone, K. C. (2006). Long-lasting changes in behavioural and neuroendocrine indices in the rat following neonatal maternal separation: gender-dependent effects. *Brain Res.* 1097, 123–132. doi: 10.1016/j.brainres.2006.04.066

Song, Z., Tai, F., Yu, C., Wu, R., Zhang, X., Broders, H., et al. (2010). Sexual or paternal experiences alter alloparental behavior and the central expression of ERα and OT in male mandarin voles (*Microtus mandarinus*). *Behav. Brain Res.* 214, 290–300. doi: 10.1016/j.bbr.2010.05.045

Trainor, B. C., Kyomen, H. H., and Marler, C. A. (2006). Estrogenic encounters: how interactions between aromatase and the environment modulate aggression. *Front. Neuroendocrinol.* 27, 170–179. doi: 10.1016/j.yfrne.2005.11.001

Trainor, B. C., and Marler, C. A. (2001). Testosterone, paternal behavior, and aggression in the monogamous California mouse *(Peromyscus californicus)*. *Horm. Behav.* 40, 32–42. doi: 10.1006/hbeh.2001.1652

Trainor, B. C., and Marler, C. A. (2002). Testosterone promotes paternal behaviour in a monogamous mammal via conversion to oestrogen. *Proc. Biol. Sci.* 269, 823–829. doi: 10.1098/rspb.2001.1954

Trainor, B. C., Takahashi, E. Y., Silva, A. L., Crean, K. K., and Hostetler, C. (2010). Sex differences in hormonal responses to social conflict in the monogamous California mouse. *Horm. Behav.* 58, 506–512. doi: 10.1016/j.yhbeh.2010.04.008

Uriarte, N., Breigeiron, M. K., Benetti, F., Rosa, X. F., and Lucion, A. B. (2007). Effects of maternal care on the development, emotionality, and reproductive functions in male and female rats. *Dev. Psychobiol.* 49, 451–462. doi: 10.1002/dev.20241

Wang, B., Li, Y., Wu, R., Zhang, S., and Tai, F. (2015). Behavioral responses to pups in males with different reproductive experiences are associated with changes in central OT, TH and OTR, D1R, D2R mRNA expression in mandarin voles. *Horm. Behav.* 67, 73–82. doi: 10.1016/j.yhbeh.2014.11.013

Wang, J., Tai, F., Yan, X., and Yu, P. (2012). Paternal deprivation alters play-fighting, serum corticosterone and the expression of hypothalamic vasopressin and oxytocin in juvenile male mandarin voles. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 198, 787–796. doi: 10.1007/s00359-012-0748-8

Weaver, I. C., Meaney, M. J., and Szyf, M. (2006). Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. *Proc. Natl. Acad. Sci. U S A* 103, 3480–3485. doi: 10.1073/pnas.0507526103

Yee, J. R., Kenkel, W. M., Frijling, J. L., Dodhia, S., Onishi, K. G., Tovar, S., et al. (2016). Oxytocin promotes functional coupling between paraventricular nucleus and both sympathetic and parasympathetic cardioregulatory nuclei. *Horm. Behav.* 80, 82–91. doi: 10.1016/j.yhbeh.2016.01.010

Yohn, C. N., Leithead, A. B., and Becker, E. A. (2017). Increased vasopressin expression in the BNST accompanies paternally induced territoriality in male and female California mouse offspring. *Horm. Behav.* 93, 9–17. doi: 10.1016/j.yhbeh.2017.03.008

Yu, P., An, S., Tai, F., Zhang, X., He, F., Wang, J., et al. (2012). The effects of neonatal paternal deprivation on pair bonding, NAcc dopamine receptor mRNA expression and serum corticosterone in mandarin voles. *Horm. Behav.* 61, 669–677. doi: 10.1016/j.yhbeh.2012.02.028

Ziegler, T. E., Wegner, F. H., Carlson, A. A., Lazaro-Perea, C., and Snowdon, C. T. (2000). Prolactin levels during the periparturitional period in the biparental cotton-top tamarin (*Saguinus oedipus*): interactions with gender androgen levels, and parenting. *Horm. Behav.* 38, 111–122. doi: 10.1006/hbeh.2000.1606

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
published: 13 February 2018
doi: 10.3389/fnbeh.2018.00020

Enduring Effects of Paternal Deprivation in California Mice (*Peromyscus californicus*): Behavioral Dysfunction and Sex-Dependent Alterations in Hippocampal New Cell Survival

Erica R. Glasper[1,2]*, *Molly M. Hyer*[2†] *and Terrence J. Hunter*[1†]

[1]*Department of Psychology, University of Maryland, College Park, College Park, MD, United States,* [2]*Program in Neuroscience and Cognitive Science, University of Maryland, College Park, College Park, MD, United States*

OPEN ACCESS

Edited by:
Martine Ammassari-Teule, Consiglio Nazionale Delle Ricerche (CNR), Italy

Reviewed by:
Francesca R. D'Amato, Consiglio Nazionale Delle Ricerche (CNR), Italy
Paul Lucassen, University of Amsterdam, Netherlands

***Correspondence:**
Erica R. Glasper
eglasper@umd.edu

†Present address:
Molly M. Hyer, Department of Anatomy and Neurobiology, Virginia Commonwealth University, Richmond, VA, United States
Terrence J. Hunter, Neuroscience Program, Tulane University, New Orleans, LA, United States

Received: *25 September 2017*
Accepted: *23 January 2018*
Published: *13 February 2018*

Citation:
Glasper ER, Hyer MM and Hunter TJ (2018) Enduring Effects of Paternal Deprivation in California Mice (Peromyscus californicus): Behavioral Dysfunction and Sex-Dependent Alterations in Hippocampal New Cell Survival. Front. Behav. Neurosci. 12:20. doi: 10.3389/fnbeh.2018.00020

Early-life experiences with caregivers can significantly affect offspring development in human and non-human animals. While much of our knowledge of parent-offspring relationships stem from mother-offspring interactions, increasing evidence suggests interactions with the father are equally as important and can prevent social, behavioral, and neurological impairments that may appear early in life and have enduring consequences in adulthood. In the present study, we utilized the monogamous and biparental California mouse (*Peromyscus californicus*). California mouse fathers provide extensive offspring care and are essential for offspring survival. Non-sibling virgin male and female mice were randomly assigned to one of two experimental groups following the birth of their first litter: (1) biparental care: mate pairs remained with their offspring until weaning; or (2) paternal deprivation (PD): paternal males were permanently removed from their home cage on postnatal day (PND) 1. We assessed neonatal mortality rates, body weight, survival of adult born cells in the dentate gyrus of the hippocampus, and anxiety-like and passive stress-coping behaviors in male and female young adult offspring. While all biparentally-reared mice survived to weaning, PD resulted in a ∼35% reduction in survival of offspring. Despite this reduction in survival to weaning, biparentally-reared and PD mice did not differ in body weight at weaning or into young adulthood. A sex-dependent effect of PD was observed on new cell survival in the dentate gyrus of the hippocampus, such that PD reduced cell survival in female, but not male, mice. While PD did not alter classic measures of anxiety-like behavior during the elevated plus maze task, exploratory behavior was reduced in PD mice. This observation was irrespective of sex. Additionally, PD increased some passive stress-coping behaviors (i.e., percent time spent immobile) during the forced swim task—an effect that was also not sex-dependent. Together, these findings demonstrate that, in a species where paternal care is not only important for offspring survival, PD can also contribute to altered structural and functional neuroplasticity of the hippocampus. The mechanisms contributing to the observed sex-dependent alterations in new cell survival in the dentate gyrus should be further investigated.

Keywords: early-life environment, paternal deprivation, sex differences, cell survival, hippocampus

INTRODUCTION

Offspring development is dependent on early bond formation with a caregiver (Rilling and Young, 2014). Lack of bond formation can result in impairments in behavior and neurodevelopmental disorders which may appear early in life (Japel et al., 1999) and persist into adulthood (Parker, 1979; Noorikhajavi et al., 2007; Tyrka et al., 2008). While the vast majority of our knowledge of parent-offspring relationships stem from mother-infant interactions (reviewed in Curley and Champagne, 2016), a few early human studies focused on the negative effects of paternal deprivation (PD) on offspring development (Green and Beall, 1962; Jensen et al., 1989). Increasing evidence from non-human animal studies suggests numerous adverse outcomes associated with PD, including dysregulated stress responses, impaired synaptic development in the prefrontal cortex, altered anxiety-like, social, and drug-seeking behaviors (Helmeke et al., 2009; Pinkernelle et al., 2009; Jia et al., 2011; Gos et al., 2014; Wang et al., 2017). Despite these advances in our knowledge, the underlying mechanisms of PD-related behavioral and neurobiological deficits remain unclear.

While fathers play a significant role in offspring care in many human societies (Kleiman and Malcolm, 1981; Hrdy, 2005), paternal, or biparental care, is rare in mammals and is observed in less than 6% of species examined (Kleiman and Malcolm, 1981). California mice (*Peromyscus californicus*) are a biparental species that are exclusively monogamous in the wild (Ribble, 1991), exhibit strong attraction and preference for the bonded mate over others (Gubernick and Nordby, 1993), and demonstrate significant paternal investment. Paternal California mice engage in many behaviors performed by the maternal female, including licking and grooming (LG), huddling, nest building, and pup retrieval (Dudley, 1974b; Gubernick and Alberts, 1987; Gubernick and Nelson, 1989; Gubernick and Nordby, 1993). This substantial investment in offspring care reduces offspring mortality and/or aids in development and growth (Dudley, 1974a; Gubernick and Alberts, 1987; Gubernick et al., 1993; Gubernick and Teferi, 2000). In the absence of the father, maternal females do not compensate for the missing paternal male (Dudley, 1974b)—an effect observed in other monogamous species as well (common degu, Helmeke et al., 2009; mandarin vole, Jia et al., 2009). Furthermore, as females of this species are highly aggressive towards conspecifics (reviewed in Steinman and Trainor, 2017), offspring care provided by multiple females is highly unlikely. Therefore, the California mouse is an excellent mouse model to investigate the consequences of PD on neurobiological outcomes.

Experiments using monogamous and biparental species suggest PD has long-lasting effects on hippocampal neurochemical systems (Wu et al., 2014; Tabbaa et al., 2017), as well as the structure and function of the hippocampus (Seidel et al., 2011; Braun et al., 2013). The hippocampus plays a key role in modulation of emotions (reviewed in Lucassen et al., 2014) and regulation of the stress response system (reviewed in Herman et al., 2016). The dentate gyrus of the hippocampus is heavily implicated in the mediation of anxiety-like behavior (Kheirbek et al., 2013; reviewed in Wu et al., 2015). More recently, a functional association between adult hippocampal neurogenesis and anxiety- and depressive-like behaviors has been demonstrated. A reduction in neurons (i.e., doublecortin positive cells; DCX+) is associated with stress-related anxiety and depressive behavior; a return to baseline DCX+ cell number results in normalization of anxiety- and depressive-like behaviors (Yun et al., 2016).

Evidence from studies using human subjects suggests that sexual dimorphisms in anxiety exist, with women largely more vulnerable than men (Kessler et al., 1994; McHenry et al., 2014). One likely underlying mechanism contributing to this sexual dimorphism in functionality of the hippocampus may be the regulation of adult neurogenesis (reviewed in Marques et al., 2016). Sex-dependent abnormalities in social- and reward-related behaviors have been observed in California mice following PD (Bambico et al., 2015) and PD increases anxiety-like behavior in adult mandarin voles (*Microtus mandarinus, Jia et al., 2009*). To what extent anxiety-like behavior and other hippocampus-related behaviors are regulated in a sex-dependent manner by PD in California mice is unknown. Therefore, the purpose of this study was to examine the interactions between sex and PD on the survival of adult born cells in the hippocampus and hippocampus-mediated behaviors, such as anxiety and passive-stress coping behavior, in young adult California mice.

MATERIALS AND METHODS

Animals

Virgin male and female California mice (60–90 days of age) were obtained from the Peromyscus Genetic Stock Center (University of South Carolina, Columbia, SC, USA) or were descendants of mice bred in our colony. Mice were provided *ad libitum* access to food and water and were housed on a 16:8 reversed light/dark cycle (lights off at 11:00 h). Non-sibling males and females were paired, allowed to mate, and give birth to their first litter. Twenty-six mating pairs resulted in 43 total offspring (**Table 1**). An average of 1.64 ± 0.58 offspring per litter were produced. On postnatal day (PND) 1 (12:00 h), two experimental groups of offspring were formed by either leaving paternal males with their mate and offspring (biparental care) or removing paternal males from the home cage (PD). This resulted in the following groups of experimental offspring: biparental care (n = male: 11; female: 10) and PD (n = male: 14; female: 8). All surviving offspring were weaned on PND 35 and housed in same-sex groups of three (i.e., some same-sex non-siblings were housed together so that individual housing of mice could be avoided). This study was carried out in accordance with guidelines provided by the National Institutes of Health for the care and use of animals. The protocol was approved by the University of Maryland Institutional Animal Care and Use Committee.

Experimental Design

On PND 60, all biparentally-reared and PD offspring were administered an intraperitoneal injection of the DNA synthesis marker bromodeoxyuridine (BrdU; 200 mg/kg; Sigma-Aldrich, St. Louis, MO, USA; cat. no. B5002) to determine the extent

TABLE 1 | Litter size and number of primiparous California mouse mate pairs.

Litter size	Number of mated pairs
1	11
2	13
3	2

to which sex and PD alter short-term survival of adult-born cells in the dentate gyrus of the hippocampus. On PND 65, all biparentally-reared and PD mice were tested for anxiety-like behavior on the elevated plus maze task (see below). On PND 67, all biparentally-reared and PD mice were assessed on a single trial version of the forced swim task, a behavioral task used to assess passive stress-coping behavior (see below). On PND 68, all mice were perfused and brain tissue was harvested in preparation for immunohistochemical processing (see below).

Elevated Plus Maze Task

Mice were individually removed from their home cages, ∼2 h after lights out, and placed in a holding cage for transportation to an adjacent behavioral room. After a 10-min acclimation period, mice were tested on the elevated plus maze under red-light illumination. The maze stood 75 cm above the floor with arms measuring 11.5 × 55 × 45 cm. Mice were placed in the center of the maze, facing an open arm, and observed for 5 min. Behavior was digitally recorded and analyzed with EthoVision®XT 11 behavioral tracking software (Noldus, Leesburg, VA, USA). Recordings were taken from a top-down view at a rate of 30 frames per second. Latency to enter the arms, duration of time spent in the arms, and number of arm entries were assessed as previously described (Glasper et al., 2015; Hyer et al., 2016). Duration of time spent in the open arms was calculated as total time spent in the open arms divided by the total time spent in both the open and closed arms, excluding the center, multiplied by 100 and presented as a percentage. Mice were returned to their holding cages immediately following the conclusion of testing and returned to the colony. Mice that froze for >40% of the time (Chauke et al., 2012) were excluded from the study (n = 7, across all groups).

Forced Swim Task

The forced swim task was performed as previously described (Hyer and Glasper, 2017). Mice were transported to the red-light illuminated behavioral room, ∼2 h after lights out, and acclimated as described above. This task consisted of placing mice in a Plexiglas cylinder (30 cm diameter, 43 cm deep) filled $\frac{3}{4}$ of the way with 23–25°C tap water for 5 min. Behavior was digitally recorded from a side view of the cylinder at 30 frames per second in an effort to distinguish between swimming and immobility behaviors (Bogdanova et al., 2013). Behavior during the task was analyzed with EthoVision®XT 11 behavioral tracking software (Noldus). The following behaviors were used to assess passive stress-coping behavior: % time immobile, latency to the first bout of immobility, and frequency of immobility bouts. Immobility was defined as mice remaining parallel to the surface of the water, only moving slightly to remain afloat. Swimming was defined as mice continuously moving paws and head. Following testing, mice were dried, warmed on a heating pad placed under their transportation cage, and returned to their home cage. Flipping behavior during the forced swim task greatly increases the likelihood that California mice will ingest water (unpublished observations); therefore, any mice that exhibited flipping behavior during the task were quickly removed and were excluded from all endpoints (n = 2, across all groups).

Histological Procedures

On PND 68, ∼2 h after lights out, mice were anesthetized using a ketamine–xylazine cocktail and transcardially perfused with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer, pH 7.0. Brains were dissected from the skull and postfixed in 4% PFA for at least 48 h at 4°C. Coronal sections (40 μm) were sliced throughout the rostrocaudal extent of the dentate gyrus on a vibrating microtome (Leica Microsystems, Chicago, IL, USA) into a bath of chilled 0.1 M phosphate-buffered saline (PBS), pH 7.5. Sections containing the dentate gyrus were identified using the Peromyscus brain atlas[1].

Immunoperoxidase Staining for BrdU

For BrdU peroxidase staining, a 1:12 series of sections were mounted onto glass Super Frost Plus slides (Fisher Scientific, Pittsburgh, PA, USA), dried, and pretreated by heating in 0.1 M citric acid, pH 6.0. Tissue was rinsed with PBS, incubated in trypsin for 10 min, denatured in 2 M HCL : PBS for 30 min, rinsed with PBS, incubated overnight in purified mouse anti-BrdU (1:200; BD Biosciences, San Jose, CA, USA; cat. no. 347580), incubated in biotinylated horse anti-mouse (1:200; Vector, Burlingame, CA, USA; cat. no. BA-2000) for 60 min, rinsed in PBS, incubated with avidin–biotin complex (Vector), rinsed with PBS, and then reacted in 0.01% diaminobenzadine with 0.003% H_2O_2. All slides were counterstained with cresyl violet, dehydrated, cleared with Citrisolv (Fisher Scientific), and coverslipped under Permount (Fisher Scientific).

Data Analysis

Quantitative analysis was conducted on coded slides. The numbers of BrdU-labeled cells on every twelfth unilateral section throughout the rostrocaudal extent of the dentate gyrus (i.e., granule cell layer, subgranular zone, and hilus) were counted at 100× magnification under oil immersion on a Zeiss Primo Star light microscope (Zeiss, Thornwood, NY, USA) using a modified version of the optical fractionator method (West et al., 1991; Ngwenya et al., 2005). The simplified formula for the estimated total number of labeled cells was: N Σ Q × (1/ssf), which is the total number of labeled cells counted (N Σ Q) multiplied by the reciprocal of the section sampling fraction (1/ssf or 1/12; Leuner et al., 2009). Brightfield photomicrographs were taken with an AxioImager camera attached to a Zeiss microscope with a stage controller using neuroimaging software (Neurolucida, Williston, VT, USA). Images were cropped and optimized by adjusting brightness and color balance in Adobe Photoshop Creative Cloud 2014.2.2.

[1]BrainMaps: An interactive multiresolution brain atlas. Available online at: http://brainmaps.org.

Statistics

Data were analyzed using GraphPad Prism version 7.03 for Windows (GraphPad Software, La Jolla, CA, USA)[2], unless otherwise noted. Survival to weaning was assessed via Log-rank (Mantel-Cox) Chi square analysis. Short-term cell survival was assessed by multiple *t*-test analysis and statistical significance was determined using the Holm-Sidak method. Two-way analysis of variance (ANOVA) was performed to assess main effects of sex and rearing condition on all behavioral endpoints (i.e., elevated plus maze, forced swim test), while three-way ANOVA was performed to assess main effects of sex, age and rearing condition on body weight using IBM® SPSS® Statistics (Version 24). Sidak's multiple comparisons tests were performed following ANOVAs, when appropriate, and the multiplicity-adjusted *p*-value was reported for each comparison. Mean differences were considered statistically significant when $p \leq 0.05$. For neuronal and behavioral analyses, final N sizes are reported within figure captions.

RESULTS

Paternal Deprivation Decreases Neonatal Survival in California Mice

We assessed the effects of PD on survival to weaning in *P. californicus* offspring. All (100%) biparentally-reared mice survived to weaning (PND 35; **Figure 1**). In contrast, PD mice displayed a marked and statistical decline in survival, with 66.67% surviving to PND 35 ($\chi^2_{(1,N=25)} = 4.96$, $p = 0.03$). By the end of the dark cycle on PND 1, greater than 20% of PD offspring perished. Between PND 1 and PND 6, an additional 15% of PD offspring were found deceased. After PND 6, no additional PD deaths were observed.

[2]www.graphpad.com

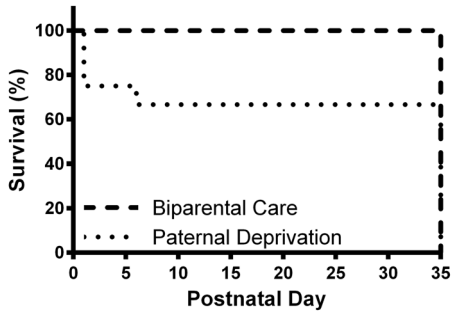

FIGURE 1 | Paternal deprivation (PD) decreases survival to weaning in California mice. Offspring were born on postnatal day (PND) 0. On PND 1, fathers remained with mate and offspring (biparental care) or were permanently removed (PD). Offspring survival was assessed daily until weaning (PND 35). All California mouse offspring reared under biparental conditions survived to weaning (dashed lines). However, by PND 1, only ∼75% of PD offspring (dotted lines) were observed alive. By PND 6, survival of PD offspring dropped to ∼65% and remained constant until weaning.

Paternal Deprivation Does Not Alter Growth of Offspring

We investigated the effects of sex, rearing, and time on body weight at numerous points during the experiment: at weaning (PND 35), at the time of BrdU injection (PND 60), and immediately before perfusion (PND 68; **Figure 2**). No interaction between sex, rearing, and time on body weight was observed ($F_{(2,66)} = 0.19$, $p = 0.83$). No interactions between rearing and time ($F_{(2,66)} = 0.10$, $p = 0.90$), sex and time ($F_{(2,66)} = 0.13$, $p = 0.88$), or sex and rearing ($F_{(2,66)} = 0.77$, $p = 0.38$) on body weight were observed. No main effects of sex ($F_{(1,66)} = 0.90$, $p = 0.35$) or rearing ($F_{(1,66)} = 1.69$, $p = 0.20$) were observed. However, a main effect of time on body weight was observed ($F_{(2,66)} = 16.40$, $p = 0.00$). Compared to weaning weight, mice weighed more at time of BrdU injection ($p = 0.00$) and time of perfusion ($p = 0.00$). No difference in weight was observed between time of BrdU injection and time of perfusion ($p = 0.53$).

Survival of Hippocampal Newborn Cells Is Reduced in Paternally-Deprived Female, but Not Male, Young Adult Offspring

The effects of PD on survival of adult born cells in the dentate gyrus of the hippocampus were investigated in young adult male and female offspring (**Figure 3**). Among males, rearing condition did not alter number of BrdU-labeled cells in the dentate gyrus ($t_{(23)} = 0.34$, $p = 0.74$). Among females, PD resulted in fewer BrdU-labeled cells in the dentate gyrus compared to biparental care ($t_{(23)} = 2.53$, $p = 0.02$).

Sex and Paternal Deprivation Alter Elevated Plus Maze Behavior in Young Adult Offspring

We examined the effects of sex and PD on anxiety-like behavior in young adult offspring, as measured by performance on the

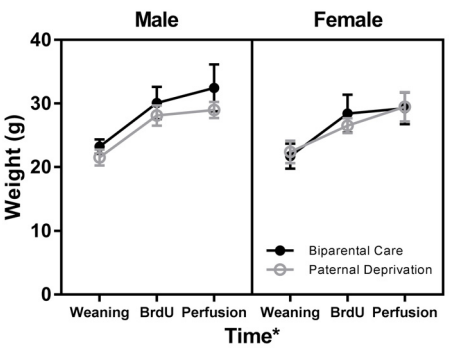

FIGURE 2 | Paternal deprivation (PD), in California mice, does not alter body weight. Male and female California mice were reared by both parents (biparental care) or by the mother alone (PD) from postnatal day (PND) 1 until weaning. Body weight was assessed at weaning (PND 35), prior to BrdU injection (PND 60), and prior to perfusion (PND 68). Compared to weaning weight, all mice, regardless of rearing condition or sex, weighed more at the time of BrdU injection and at perfusion. Symbols represent mean ± SEM. *, main effect of time, $p \leq 0.05$.

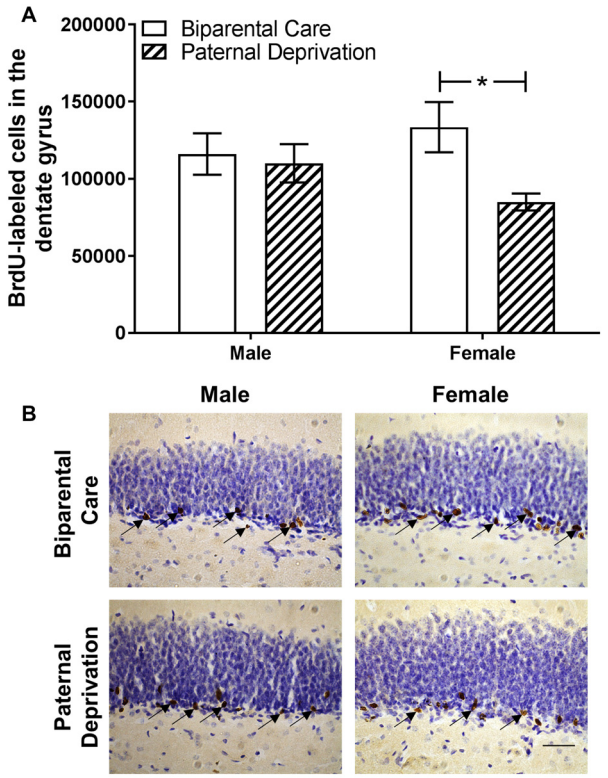

FIGURE 3 | Paternal deprivation (PD) reduces new cell survival in the hippocampus of female, but not male, young adult California mouse offspring. **(A)** Short-term survival of bromodeoxyuridine (BrdU)-labeled cells was determined in California mouse young adult male and female offspring reared by both parents (biparental care) or reared by mother alone (PD) from postnatal day (PND) 1 to weaning PD. PD reduced the number of BrdU-labeled cells in the dentate gyrus of female, but not male, offspring. N sizes: biparental male, 6; biparental female, 6; PD male, 9; PD female, 6. Bars represent mean ± SEM. $^*p \leq 0.05$. **(B)** Representative photomicrographs (40× oil magnification) of BrdU-labeled cells in the dentate gyrus of the hippocampus. Scale bar = 40 μm. Arrows point to BrdU-labeled cells.

elevated plus maze task. In % time spent on the open arms (**Figure 4A**), a significant interaction between rearing and sex was observed ($F_{(1,19)} = 5.89$, $p = 0.03$). Among biparentally-reared mice, females spent considerably more time on the open arms than males ($p = 0.03$); however, this sex effect was not observed in PD mice ($p = 0.71$). No main effect of sex ($F_{(1,19)} = 1.95$, $p = 0.18$) or rearing ($F_{(1,19)} = 0.03$, $p = 0.87$) was observed in % time on the open arms. Latency to enter the open arms (**Figure 4B**) was not altered by rearing ($F_{(1,19)} = 0.000$, $p = 0.98$) or sex ($F_{(1,19)} = 0.16$, $p = 0.69$) and no interaction between rearing and sex was observed ($F_{(1,19)} = 2.22$, $p = 0.69$). Total arm entries (**Figure 4C**) were not altered by rearing ($F_{(1,19)} = 1.69$, $p = 0.21$) or sex ($F_{(1,19)} = 0.02$, $p = 0.88$) and no interaction between rearing and sex was detected ($F_{(1,19)} = 0.34$, $p = 0.57$). In total distance traveled (**Figure 4D**), a main effect of rearing ($F_{(1,19)} = 6.40$, $p = 0.02$), but not sex ($F_{(1,19)} = 2.53$, $p = 0.13$), was observed. Overall, PD decreased the total distance traveled during the elevated plus maze task. No interaction between rearing and sex ($F_{(1,19)} = 0.39$, $p = 0.54$) was observed.

Paternal Deprivation and Sex Alter Passive Stress-Coping Behavior in Young Adult Offspring

We determined the effects of PD and sex on passive stress-coping behavior, during the forced swim task, in young adult male and female offspring. In % time immobile (**Figure 5A**), a main effect of rearing ($F_{(1,22)} = 4.72$, $p = 0.04$), but not sex ($F_{(1,22)} = 1.93$, $p = 0.18$), was observed. Overall, PD increased % time spent immobile during the forced swim task. No interaction between rearing and sex was observed in % time immobile ($F_{(1,22)} = 0.00$, $p = 0.97$). Latency to the first bout of immobility (**Figure 5B**) was significantly altered by sex ($F_{(1,22)} = 29.54$, $p < 0.00$) but not rearing ($F_{(1,22)} = 0.05$, $p = 0.83$). Males, irrespective of rearing, displayed passive stress-coping behavior (i.e., floating) faster than females. No interaction between rearing and sex was observed ($F_{(1,22)} = 0.25$, $p = 0.62$). Bouts of immobility (**Figure 5C**) were also not altered by rearing ($F_{(1,22)} = 2.72$, $p = 0.11$) or sex ($F_{(1,22)} = 0.12$, $p = 0.73$), and no interaction between rearing and sex was observed ($F_{(1,22)} = 2.22$, $p = 0.15$).

DISCUSSION

In the present study, we demonstrated that PD in *P. californicus*, a biparental mouse species, is associated with reduced survival during early postnatal development, sex-dependent deficits in hippocampal structural plasticity, reduced exploratory behavior, and impaired stress coping in young adulthood. PD results in ∼35% decrease in offspring survival to weaning. Of those offspring that survive to weaning, no differences in body weight are detected; however, a sex-dependent decrease in the number of adult-born cells is observed in the dentate gyrus of the hippocampus. Specifically, PD females, but not males, exhibit reduced short-term survival of newborn cells in the dentate gyrus. Additionally, PD decreases exploratory behavior, but not classic anxiety-like behaviors, during the elevated plus maze task. Notably, and for the first time shown here, PD increases some measures of passive stress-coping behavior during the forced swim task (i.e., % time immobile). Together, these findings suggest that the lack of paternal care, in a biparental species, may contribute to long-lasting effects on structural plasticity and behavioral function of the hippocampus.

California mouse fathers spend more time interacting with offspring during the early, compared to late, postnatal period (Bester-Meredith et al., 1999). Here, removing the paternal male from the home cage resulted in a significant decline in early (i.e., PND 1) postnatal survival. As previously mentioned, California mice are an excellent model of biparental care given the significant paternal care provided by California mouse fathers. Male and female California mice parents spend similar amounts of time in the nest (Dudley, 1974a) and aside from nursing, parental behaviors performed on PND 1 are shared equally by both the mother and father. Specifically, California mouse fathers and mothers spend equal amounts of time in the nest as well as equivalent durations of time in physical contact with the pups (Gubernick and Alberts, 1987). On the whole,

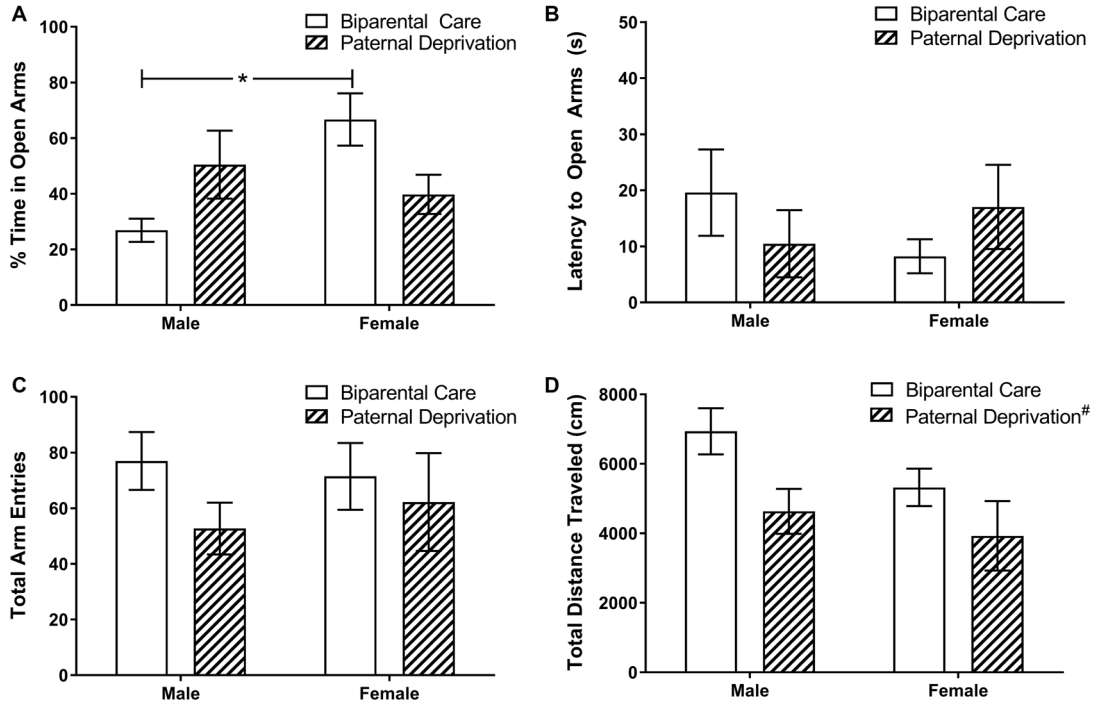

FIGURE 4 | Classic indices of anxiety-like behavior in the elevated plus maze are not altered by paternal deprivation (PD) among young adult California mice. Male and female California mice were reared by both parents (biparental care) or by the mother alone (PD) from postnatal day (PND) 1 until weaning. On PND 65, mice were tested on the elevated plus maze task for 5 min. **(A)** Among biparentally-reared offspring, females spent considerably more time on the open arms than males. PD did not alter time spent on the open arms. $^*p \leq 0.05$. **(B,C)** Neither sex, nor rearing, altered latency to enter the open arms or total arm entries. **(D)** Irrespective of sex, total distance traveled within the elevated plus maze was reduced by PD. N sizes: biparental care male; 4; biparental care female, 7; PD male, 7; PD female, 5. Bars represent mean ± SEM. #, main effect of PD.

the early parental behaviors performed by fathers are similar to that performed by mothers, however, fathers do perform more non-anogenital licking of pups than mothers on PND 1 (Gubernick and Alberts, 1987).

When dead pups were observed, they were either cannibalized or found unaltered outside of the nest, an outcome previously reported in studies of California mice (Gubernick et al., 1993). Pup death may be mediated, in part, by the dam's response to the absence of her mate. It is not uncommon for maternal California mice to cannibalize or withhold care from young following mate disappearance (Gubernick et al., 1993). A rapid termination of the dam's reproductive investments following the removal of the mate may reflect the inability to successfully rear pups alone, an effect also observed in the monogamous, biparental Djungarian hamster (*Phodopus cambelli*); all pups observed deceased 3 days postpartum if paternal male is removed (Wynne-Edwards, 1987; Wynne-Edwards and Lisk, 1989). In the current study, PD offspring survived if they lived to PND 6. Interestingly, if California mice parents decide to forgo offspring care, pups are observed deceased 2–5 days following birth (Cantoni and Brown, 1997). It should also be noted that offspring survival is decreased even when males are removed several days before the birth of pups (Gubernick et al., 1993), therefore the increase in offspring mortality is likely not a result of experimenter handling or nest disruption.

Increased pup death may also be due to problems related to thermoregulation and/or metabolism. Thermoregulation, in California mice pups, is related to the presence of the father (Dudley, 1974b); individual California mice offspring are ectothermic prior to PND 15 (Gubernick, 1987). Given that most of the male's early parental care is in the form of huddling over pups (Gubernick and Alberts, 1987), direct male care may enhance offspring survival by providing warmth, as previously described (Dudley, 1974a). Heat transfer may be even more necessary under harsh environmental conditions, such as cold temperatures and/or when foraging for food is necessary (Gubernick et al., 1993; Wright and Brown, 2002; Bredy et al., 2007). It is likely that under harsh laboratory conditions, our survival rate would be lower than what is reported in the current study. In addition to thermoregulation, problems associated with metabolism may contribute to offspring mortality. Following mate removal, California mouse mothers stop lactating 5–28 days later (Gubernick and Teferi, 2000). It is possible that PD offspring receive less nourishment than biparentally-reared offspring, which may ultimately contribute to increased mortality. Future studies are necessary to determine to what extent increased California mouse mortality, following the removal of the paternal male, is a result of the direct absence of paternal care, since California mice dams do not overcompensate for their partners'

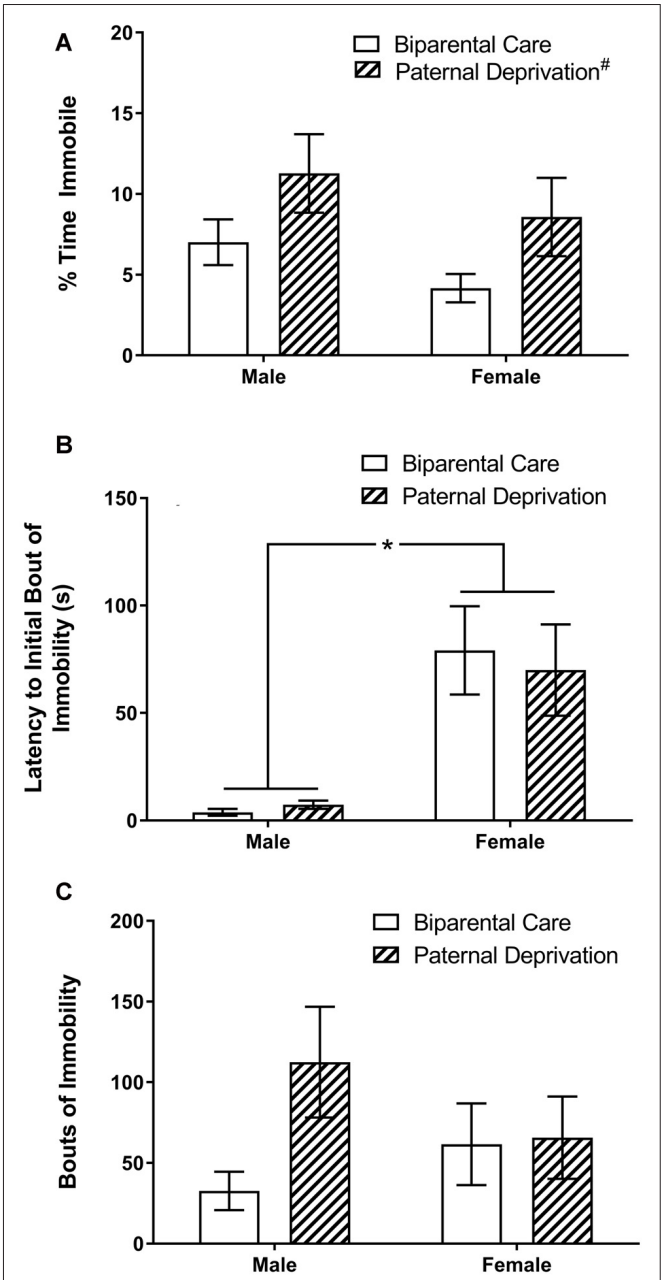

FIGURE 5 | Both rearing and sex alter indices of passive stress-coping behavior during the forced swim test in young adult California mice. Male and female California mice were reared by both parents (biparental care) or by the mother alone [paternal deprivation (PD)] from postnatal day (PND) 1 until weaning. On PND 67, mice underwent a 5 min forced swim test. **(A)** Overall, PD increases % time immobile. #, main effect of PD. **(B)** Irrespective of rearing, males display immobility faster than females. $^*p \leq 0.05$. **(C)** Neither sex, nor rearing, alter bouts of immobility. N sizes: biparental care male, 8; biparental care female, 7; PD male, 5; PD female, 6. Bars represent mean ± SEM.

absence (Dudley, 1974b), or indirect effects of altered maternal care. Recent evidence from our lab has demonstrated no differences in pup retrieval between multiparous California mouse mothers rearing pups with or without her mate (Madison et al., 2017). Pup retrieval is only one of many maternal behaviors, therefore a thorough analysis of parental behavior should be performed.

A sex-dependent effect of PD on the short-term survival of adult born cells in the dentate gyrus of the hippocampus was observed. Specifically, PD females exhibited a marked decline in the number of BrdU-labeled cells, compared to females reared by both parents. No effect of PD was observed in the short-term survival of adult born cells in males. While the phenotype of these 8-day old cells was not assessed in the current study, doublecortin (DCX) is expressed in the majority (∼89%) of 1-week old BrdU-labeled cells in the hippocampus of young adult mice (Snyder et al., 2009). DCX is expressed in young neurons as well as neuronal precursors and plays key roles in neuronal maturation (Brown et al., 2003; Kerjan et al., 2009). This sex-dependent effect of PD on short-term cell survival in the dentate gyrus of the hippocampus aligns with sex-dependent effects of PD on neuroendocrine regulation and brain neurochemistry observed in biparental rodents, including the California mouse. Serum corticosterone and adrenocorticotrophin concentrations are increased in adult female, but not male, mandarin voles exposed to PD (Wu et al., 2014). Since stress and elevated glucocorticoids have been repeatedly shown to inhibit new cell production and survival (reviewed in Mirescu and Gould, 2006), the decreased cell survival in our PD females, but not males, may reflect baseline differences in serum corticosterone. This was not assessed in our current study and should be further explored. Decreased hippocampal glucocorticoid receptor (GR) and brain-derived neurotrophic factor (BDNF) have been shown in female, but not male, mandarin voles (Wu et al., 2014). Additionally, an attenuation in basal activity of low-spiking medial prefrontal cortex pyramidal cells has been observed in female, but not male, California mice (Bambico et al., 2015). In male, but not female, degus (*Octodon degus*), PD results in early (i.e., time of weaning) deficits in dendritic plasticity of the orbitofrontal cortex—an effect that is no longer apparent in adulthood (Helmeke et al., 2009). It is unknown whether similar developmental trajectories exist in hippocampal structural plasticity in male California mice.

It is important to note that other models of early-life stress, (i.e., disrupted maternal care) in uniparental species, like mice and rats, results in sex-dependent alterations to hippocampal neuroplasticity. Twenty-four hours of maternal deprivation on PND 3 does not alter anxiety-like behavior, cognitive function, or adult neurogenesis in 12–17 week old female rats (Loi et al., 2017) but does lead to accelerated maturation of synaptic plasticity in male rats (Derks et al., 2016). At weaning (PND 21), maternal deprivation results in increased immature neuron survival (i.e., DCX+ cells) in male rats and decreased DCX+ cells in female rats. This effect was likely not driven by enduring sex-dependent changes in maternal behavior following maternal deprivation on PND 3 (Oomen et al., 2009). Maternal deprivation ∼1 week later (i.e., PND 9) immunologically primes hippocampal synapses of male, but not female, juvenile rats (Viviani et al., 2014); reduced anxiety-like behavior and increased risk taking behaviors are observed in females only (Mela et al., 2015). Females may be more resilient than males to the effects of early-life stress (Walker et al., 2011), as early life nest and bedding disruption models describe

enduring negative consequences in male mice only, including increased basal corticosterone concentrations, decreased spatial and object recognition memory, and decreased hippocampal adult neurogenesis (Rice et al., 2008; Naninck et al., 2015). To what extent similar sex-dependent observations occur, as a result of maternal deprivation, in a biparental mammalian species has yet to be investigated. Interestingly, in the biparental zebra finch (*Taeniopygia guttata*), maternal deprivation results in hyperresponsivity to stress and altered mRNA levels of GR and mineralocorticoid receptors in the hippocampus, cerebellum, and hypothalamus (Banerjee et al., 2012).

To what extent the lack of direct paternal care mediates the observed sex-dependent effects on new cell survival is unclear, however, it is conceivable that differential distribution of parental care may play a factor. California mouse fathers engage in more pup licking than mothers; however, fathers spend more time licking non-anogenital regions compared to mothers (Gubernick and Alberts, 1987). In rats, mothers engage in more anogenital licking of male, compared to female, offspring (Richmond and Sachs, 1984). This attentional bias toward male offspring could have long-term consequences on development (Moore and Power, 1992). While this bias in anogenital licking has not been reported in California mice, it is possible that male offspring receive more direct care from the mother in the form of anogenital licking, thereby providing more parental care, thus preventing a decline in hippocampal structural plasticity. More detailed analysis of home cage parental behavior following PD may shed light on this possibility given that maternal deprivation on PND 3 results in greater LG on PND 4 in rat offspring, with males receiving more attention than females (Oomen et al., 2009); however, both the sex difference in LG and overall increase in LG disappears by PND 5. To what extent similar findings are observed following PD in California mice should be further explored.

We did not observe an overall anxious phenotype among PD offspring. During elevated plus maze testing, classical indices of anxiety like behavior (i.e., reduced % time in the open arms, increased latency to enter open arms; Komada et al., 2008) were not observed following PD. However, exploratory behavior (i.e., total distance traveled) was reduced in both male and female PD offspring, compared to offspring receiving biparental care. A reduction in locomotor activity, without altered anxiety-like behavior, has been observed during assessments of anxiety-like behavior in PD mandarin voles (Jia et al., 2009; Tabbaa et al., 2017). In fact, reduced locomotor activity following PD has been observed in both rodent and non-human primate species (Dettling et al., 2002; Cao et al., 2014). Collectively, these studies of PD in various mammalian species suggest that decreased exploratory behavior may be indicative of an anxious phenotype (Kõks et al., 1997) that may complicate more traditional indices of anxiety-like behavior on the elevated plus maze. Despite the lack of a sex-dependent effect in anxiety-like behavior within the PD group, contrasted with the effect observed within the biparental care group, restraint should be taken when interpreting the effects of PD on anxiety-like behavior when exploration is a primary component of the behavioral task (e.g., elevated plus maze).

PD, independent of sex, resulted in increased total time spent immobile during the forced swim task. This is the first demonstration, to our knowledge, of increased passive stress-coping behavior following PD. Chronic physical and psychological stressors significantly alter regulation of neuroendocrine systems and reorganize brain regions, like the hippocampus, which are highly responsive to stress hormones (i.e., corticosterone; reviewed in de Kloet and Molendijk, 2016). It is plausible that PD altered neuroendocrine regulation, yet this was not assessed in the current study. Increased basal corticosterone has been observed throughout the postpartum period (Wang et al., 2014) and at weaning (Wang et al., 2012) in mandarin vole offspring following removal of the paternal male on PND 0 (i.e., day of birth). Mice with a history of stress, followed by exposure to the forced swim task, exhibit upregulation of genes in the hippocampus that are involved in chromatin modification and epigenetics (e.g., BDNF and GR). The altered expression of some of these genes can be long-lasting (Gray et al., 2014; Hashikawa et al., 2015) and may underlie immobility behavior during the forced swim task (De Pablo et al., 1989; Campus et al., 2015). Latency to immobility, or floating, is considered a main outcome measure of the forced swim task. In the current experiment, the time from placement in the cylinder to the first bout of immobility was markedly faster among males than females. This effect was independent of rearing. However, the total number of immobility bouts did not differ as a result of sex. Therefore, although male California mice exhibited earlier passive stress-coping behavior than females, this sex difference did not influence global performance in the forced swim task.

In summary, our findings highlight the consequences of PD in a biparental rodent species, the California mouse. Removal of the father was associated with reduced structural plasticity among female mice and generalized deficits in exploratory and passive-stress coping behaviors. In humans, quality, rather than continuity, of parental care is associated with impaired behavioral dysfunction (i.e., depression; Parker, 1979). Given that maternal California mice do not compensate for missing paternal contributions (Dudley, 1974a; Bester-Meredith and Marler, 2003), the quality of care received by PD offspring may be reduced, resulting in enduring effects on hippocampal neuroplasticity and even survival. Mechanisms underlying sex-differences in short-term survival should be explored. Additionally, future studies should investigate to what extent these findings are a direct result of paternal removal or an indirect result of altered maternal care following mate removal.

AUTHOR CONTRIBUTIONS

ERG: substantial contribution to the conception of the work; critically revising the work for intellectual content. ERG and MMH: substantial contribution to the design of the work. MMH and TJH: acquisition of data for the work. ERG, MMH and TJH: analysis of the data for the work, interpretation of the data for the work and final approval of the version to be published. ERG and TJH: drafting the work. ERG: critically revising the work for intellectual content.

ACKNOWLEDGMENTS

This work was supported by a Research and Service Award from the Graduate School at the University of Maryland (ERG).

Many thanks to Cecily Greene for expert animal care. Additional contributions (e.g., data collection, image acquisition, reading early drafts) provided by Nathan Luke, Jhansi Khatakam, Robyn Harper, Luke Hallgarth, and Sabina Khantsis.

REFERENCES

Bambico, F. R., Lacoste, B., Hattan, P. R., and Gobbi, G. (2015). Father absence in the monogamous california mouse impairs social behavior and modifies dopamine and glutamate synapses in the medial prefrontal cortex. *Cereb. Cortex* 25, 1163–1175. doi: 10.1093/cercor/bht310

Banerjee, S. B., Arterbery, A. S., Fergus, D. J., and Adkins-Regan, E. (2012). Deprivation of maternal care has long-lasting consequences for the hypothalamic-pituitary-adrenal axis of zebra finches. *Proc. Biol. Sci.* 279, 759–766. doi: 10.1098/rspb.2011.1265

Bester-Meredith, J. K., and Marler, C. A. (2003). Vasopressin and the transmission of paternal behavior across generations in mated, cross-fostered Peromyscus mice. *Behav. Neurosci.* 117, 455–463. doi: 10.1037/0735-7044.117.3.455

Bester-Meredith, J. K., Young, L. J., and Marler, C. A. (1999). Species differences in paternal behavior and aggression in peromyscus and their associations with vasopressin immunoreactivity and receptors. *Horm. Behav.* 36, 25–38. doi: 10.1006/hbeh.1999.1522

Bogdanova, O. V., Kanekar, S., D'Anci, K. E., and Renshaw, P. F. (2013). Factors influencing behavior in the forced swim test. *Physiol. Behav.* 118, 227–239. doi: 10.1016/j.physbeh.2013.05.012

Braun, K., Seidel, K., Holetschka, R., Groeger, N., and Poeggel, G. (2013). Paternal deprivation alters the development of catecholaminergic innervation in the prefrontal cortex and related limbic brain regions. *Brain Struct. Funct.* 218, 859–872. doi: 10.1007/s00429-012-0434-1

Bredy, T. W., Brown, R. E., and Meaney, M. J. (2007). Effect of resource availability on biparental care and offspring neural and behavioral development in the California mouse (*Peromyscus californicus*). *Eur. J. Neurosci.* 25, 567–575. doi: 10.1111/j.1460-9568.2006.05266.x

Brown, J. P., Couillard-Després, S., Cooper-Kuhn, C. M., Winkler, J., Aigner, L., and Kuhn, H. G. (2003). Transient expression of doublecortin during adult neurogenesis. *J. Comp. Neurol.* 467, 1–10. doi: 10.1002/cne.10874

Campus, P., Colelli, V., Orsini, C., Sarra, D., and Cabib, S. (2015). Evidence for the involvement of extinction-associated inhibitory learning in the forced swimming test. *Behav. Brain Res.* 278, 348–355. doi: 10.1016/j.bbr.2014.10.009

Cantoni, D., and Brown, R. E. (1997). Paternal investment and reproductive success in the California mouse, *Peromyscus californicus*. *Anim. Behav.* 54, 377–386. doi: 10.1006/anbe.1996.0583

Cao, Y., Wu, R., Tai, F., Zhang, X., Yu, P., An, X., et al. (2014). Neonatal paternal deprivation impairs social recognition and alters levels of oxytocin and estrogen receptor α mRNA expression in the MeA and NAcc and serum oxytocin in mandarin voles. *Horm. Behav.* 65, 57–65. doi: 10.1016/j.yhbeh.2013.11.005

Chauke, M., de Jong, T. R., Garland, T., and Saltzman, W. (2012). Paternal responsiveness is associated with, but not mediated by reduced neophobia in male California mice (*Peromyscus californicus*). *Physiol. Behav.* 107, 65–75. doi: 10.1016/j.physbeh.2012.05.012

Curley, J. P., and Champagne, F. A. (2016). Influence of maternal care on the developing brain: mechanisms, temporal dynamics and sensitive periods. *Front. Neuroendocrinol.* 40, 52–66. doi: 10.1016/j.yfrne.2015.11.001

de Kloet, E. R., and Molendijk, M. L. (2016). Coping with the forced swim stressor: towards understanding an adaptive mechanism. *Neural Plast.* 2016:6503162. doi: 10.1155/2016/6503162

De Pablo, J. M., Parra, A., Segovia, S., and Guillamón, A. (1989). Learned immobility explains the behavior of rats in the forced swimming test. *Physiol. Behav.* 46, 229–237. doi: 10.1016/0031-9384(89)90261-8

Derks, N. A. V., Krugers, H. J., Hoogenraad, C. C., Joëls, M., and Sarabdjitsingh, R. A. (2016). Effects of early life stress on synaptic plasticity in the developing hippocampus of male and female rats. *PLoS One* 11:e0164551. doi: 10.1371/journal.pone.0164551

Dettling, A. C., Feldon, J., and Pryce, C. R. (2002). Early deprivation and behavioral and physiological responses to social separation/novelty in the marmoset. *Pharmacol. Biochem. Behav.* 73, 259–269. doi: 10.1016/s0091-3057(02)00785-2

Dudley, D. (1974a). Contributions of paternal care to the growth and development of the young in *Peromyscus californicus*. *Behav. Biol.* 11, 155–166. doi: 10.1016/s0091-6773(74)90305-8

Dudley, D. (1974b). Paternal behavior in the California mouse, *Peromyscus californicus*. *Behav. Biol.* 11, 247–252. doi: 10.1016/s0091-6773(74)90433-7

Glasper, E. R., Hyer, M. M., Katakam, J., Harper, R., Ameri, C., and Wolz, T. (2015). Fatherhood contributes to increased hippocampal spine density and anxiety regulation in California mice. *Brain Behav.* 6:e00416. doi: 10.1002/brb3.416

Gos, T., Schulkin, J., Gos, A., Bock, J., Poeggel, G., and Braun, K. (2014). Paternal deprivation affects the functional maturation of corticotropin-releasing hormone (CRH)- and calbindin-D28k-expressing neurons in the bed nucleus of the stria terminalis (BNST) of the biparental Octodon degus. *Brain Struct. Funct.* 219, 1983–1990. doi: 10.1007/s00429-013-0617-4

Gray, J. D., Rubin, T. G., Hunter, R. G., and McEwen, B. S. (2014). Hippocampal gene expression changes underlying stress sensitization and recovery. *Mol. Psychiatry* 19, 1171–1178. doi: 10.1038/mp.2013.175

Green, M., and Beall, P. (1962). A report of nineteen cases: paternal deprivation-a disturbance in fathering. *Pediatrics* 30, 91–99.

Gubernick, D. J. (1987). Ontogeny of thermoregulation in the California mouse. *Unpublished Raw Data.*

Gubernick, D. J., and Alberts, J. R. (1987). The biparental care system of the California mouse, *Peromyscus californicus*. *J. Comp. Psychol.* 101, 169–177. doi: 10.1037/0735-7036.101.2.169

Gubernick, D. J., and Nelson, R. J. (1989). Prolactin and paternal behavior in the biparental California mouse, *Peromyscus californicus*. *Horm. Behav.* 23, 203–210. doi: 10.1016/0018-506x(89)90061-5

Gubernick, D. J., and Nordby, J. C. (1993). Mechanisms of sexual fidelity in the monogamous California mouse, *Peromyscus californicus*. *Behav. Ecol. Sociobiol.* 32, 211–219. doi: 10.1007/bf00173779

Gubernick, D. J., and Teferi, T. (2000). Adaptive significance of male parental care in a monogamous mammal. *Proc. Biol. Sci.* 267, 147–150. doi: 10.1098/rspb.2000.0979

Gubernick, D. J., Wright, S. L., and Brown, R. E. (1993). The significance of the father's presence for offspring survival in the monogamous California mouse, *Peromyscus californicus*. *Anim. Behav.* 46, 539–546. doi: 10.1006/anbe.1993.1221

Hashikawa, N., Ogawa, T., Sakamoto, Y., Ogawa, M., Matsuo, Y., Zamami, Y., et al. (2015). Time course of behavioral alteration and mRNA levels of neurotrophic factor following stress exposure in mouse. *Cell. Mol. Neurobiol.* 35, 807–817. doi: 10.1007/s10571-015-0174-x

Helmeke, C., Seidel, K., Poeggel, G., Bredy, T. W., Abraham, A., and Braun, A. K. (2009). Paternal deprivation during infancy results in dendrite- and time-specific changes of dendritic development and spine formation in the orbitofrontal cortex of the biparental rodent Octodon degus. *Neuroscience* 163, 790–798. doi: 10.1016/j.neuroscience.2009.07.008

Herman, J. P., McKlveen, J. M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., et al. (2016). Regulation of the hypothalamic-pituitary-adrenocortical stress response. *Compr. Physiol.* 6, 603–621. doi: 10.1002/cphy.c150015

Hrdy, S. B. (2005). "Evolutionary context of human development: the cooperative breeding model," in *Attachment and Bonding. A New Synthesis*, eds. C. S. Carter, L. Ahnert, K. E. Grossmann, S. B. Hrdy, M. E. Lamb and S. W. Porges (Cambridge, MA: MIT Press), 9–32.

Hyer, M. M., and Glasper, E. R. (2017). Separation increases passive stress-coping behaviors during forced swim and alters hippocampal dendritic morphology in California mice. *PLoS One* 12:e0175713. doi: 10.1371/journal.pone.0175713

Hyer, M. M., Hunter, T. J., Katakam, J., Wolz, T., and Glasper, E. R. (2016). Neurogenesis and anxiety-like behavior in male California mice during the mate's postpartum period. *Eur. J. Neurosci.* 43, 703–709. doi: 10.1111/ejn.13168

Japel, C., Tremblay, R. E., Vitaro, F., and Boulerice, B. (1999). Early parental separation and the psychosocial development of daughters 6-9 years old. *Am. J. Orthopsychiatry* 69, 49–60. doi: 10.1037/h0080381

Jensen, P. S., Grogan, D., Xenakis, S. N., and Bain, M. W. (1989). Father absence: effects on child and maternal psychopathology. *J. Am. Acad. Child Adolesc. Psychiatry* 28, 171–175. doi: 10.1097/00004583-198903000-00004

Jia, R., Tai, F., An, S., Zhang, X., and Broders, H. (2009). Effects of neonatal paternal deprivation or early deprivation on anxiety and social behaviors of the adults in mandarin voles. *Behav. Processes* 82, 271–278. doi: 10.1016/j.beproc.2009.07.006

Jia, R., Tai, F., An, S., and Zhang, X. (2011). Neonatal paternal deprivation or early deprivation reduces adult parental behavior and central estrogen receptor α expression in mandarin voles (*Microtus mandarinus*). *Behav. Brain Res.* 224, 279–289. doi: 10.1016/j.bbr.2011.05.042

Kerjan, G., Koizumi, H., Han, E. B., Dubé, C. M., Djakovic, S. N., Patrick, G. N., et al. (2009). Mice lacking doublecortin and doublecortin-like kinase 2 display altered hippocampal neuronal maturation and spontaneous seizures. *Proc. Natl. Acad. Sci. U S A* 106, 6766–6771. doi: 10.1073/pnas.0812687106

Kessler, R. C., McGonagle, K. A., Zhao, S., Nelson, C. B., Hughes, M., Eshleman, S., et al. (1994). Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the national comorbidity survey. *Arch. Gen. Psychiatry* 51, 8–19. doi: 10.1001/archpsyc.1994.03950010008002

Kheirbek, M. A., Drew, L. J., Burghardt, N. S., Costantini, D. O., Tannenholz, L., Ahmari, S. E., et al. (2013). Differential control of learning and anxiety along the dorsoventral axis of the dentate gyrus. *Neuron* 77, 955–968. doi: 10.1016/j.neuron.2012.12.038

Kleiman, D. G., and Malcolm, J. R. (1981). "The evolution of male parental investment in mammals," in *Parental Care in Mammals*, eds. D. J. Gubernick and P. H. Klopfer (New York, NY: Plenum Press), 347–387.

Kõks, S., Vasar, E., Soosaar, A., Lang, A., Volke, V., Võikar, V., et al. (1997). Relation of exploratory behavior of rats in elevated plus-maze to brain receptor binding properties and serum growth hormone levels. *Eur. Neuropsychopharmacol.* 7, 289–294. doi: 10.1016/s0924-977x(97)00034-5

Komada, M., Takao, K., and Miyakawa, T. (2008). Elevated plus maze for mice. *J. Vis. Exp.* 22:1088. doi: 10.3791/1088

Leuner, B., Glasper, E. R., and Gould, E. (2009). Thymidine analog methods for studies of adult neurogenesis are not equally sensitive. *J. Comp. Neurol.* 517, 123–133. doi: 10.1002/cne.22107

Loi, M., Mossink, J. C. L., Meerhoff, G. F., Den Blaauwen, J. L., Lucassen, P. J., and Joëls, M. (2017). Effects of early-life stress on cognitive function and hippocampal structure in female rodents. *Neuroscience* 342, 101–119. doi: 10.1016/j.neuroscience.2015.08.024

Lucassen, P. J., Pruessner, J., Sousa, N., Almeida, O. F. X., Van Dam, A. M., Rajkowska, G., et al. (2014). Neuropathology of stress. *Acta Neuropathol.* 127, 109–135. doi: 10.1007/s00401-013-1223-5

Madison, F. N., Whitaker, A. R., Khantsis, S., and Glasper, E. R. (2017). "Sex differences in early postnatal ultrasonic vocalizations and adult hippocampal microglial morphology in California mice (*Peromyscus californicus*) exposed to paternal deprivation," in *Neuroscience Meeting Planner* (Washington, DC: Society for Neuroscience).

Marques, A. A., Bevilaqua, M. C. D. N., da Fonseca, A. M. P., Nardi, A. E., Thuret, S., and Dias, G. P. (2016). Gender differences in the neurobiology of anxiety: Focus on adult hippocampal neurogenesis. *Neural Plast.* 2016:5026713. doi: 10.1155/2016/5026713

McHenry, J., Carrier, N., Hull, E., and Kabbaj, M. (2014). Sex differences in anxiety and depression: role of testosterone. *Front. Neuroendocrinol.* 35, 42–57. doi: 10.1016/j.yfrne.2013.09.001

Mela, V., Díaz, F., Borcel, E., Argente, J., Chowen, J. A., and Viveros, M. P. (2015). Long term hippocampal and cortical changes induced by maternal deprivation and neonatal Leptin treatment in male and female rats. *PLoS One* 10:e0137283. doi: 10.1371/journal.pone.0137283

Mirescu, C., and Gould, E. (2006). Stress and adult neurogenesis. *Hippocampus* 16, 233–238. doi: 10.1002/hipo.20155

Moore, C. L., and Power, K. L. (1992). Variation in maternal care and individual differences in play, exploration, and grooming of juvenile Norway rat offspring. *Dev. Psychobiol.* 25, 165–182. doi: 10.1002/dev.420250303

Naninck, E. F. G., Hoeijmakers, L., Kakava-Georgiadou, N., Meesters, A., Lazic, S. E., Lucassen, P. J., et al. (2015). Chronic early life stress alters developmental and adult neurogenesis and impairs cognitive function in mice. *Hippocampus* 25, 309–328. doi: 10.1002/hipo.22374

Ngwenya, L. B., Peters, A., and Rosene, D. L. (2005). Light and electron microscopic immunohistochemical detection of bromodeoxyuridine-labeled cells in the brain: different fixation and processing protocols. *J. Histochem. Cytochem.* 53, 821–832. doi: 10.1369/jhc.4a6605.2005

Noorikhajavi, M., Afghah, S., Dadkhah, A., Holakoyie, K., and Motamedi, S. H. (2007). The effect of "parental loss" under 18 on developing "MDD" in adult age. *Int. J. Psychiatry Med.* 37, 347–355. doi: 10.2190/PM.37.3.j

Oomen, C. A., Girardi, C. E. N., Cahyadi, R., Verbeek, E. C., Krugers, H., Joëls, M., et al. (2009). Opposite effects of early maternal deprivation on neurogenesis in male versus female rats. *PLoS One* 4:e3675. doi: 10.1371/journal.pone.0003675

Parker, G. (1979). Parental deprivation and depression in a non-clinical group. *Aust. N Z J. Psychiatry* 13, 51–56. doi: 10.3109/00048677909159109

Pinkernelle, J., Abraham, A., Seidel, K., and Braun, K. (2009). Paternal deprivation induces dendritic and synaptic changes and hemispheric asymmetry of pyramidal neurons in the somatosensory cortex. *Dev. Neurobiol.* 69, 663–673. doi: 10.1002/dneu.20726

Ribble, D. O. (1991). The monogamous mating system of *Peromyscus californicus* as revealed by DNA finger-printing. *Behav. Ecol. Sociobiol.* 29, 161–166. doi: 10.1007/bf00166397

Rice, C. J., Sandman, C. A., Lenjavi, M. R., and Baram, T. Z. (2008). A novel mouse model for acute and long-lasting consequences of early life stress. *Endocrinology* 149, 4892–4900. doi: 10.1210/en.2008-0633

Richmond, G., and Sachs, B. D. (1984). Maternal discrimination of pup sex in rats. *Dev. Psychobiol.* 17, 87–89. doi: 10.1002/dev.420170108

Rilling, J. K., and Young, L. J. (2014). The biology of mammalian parenting and its effect on offspring social development. *Science* 345, 771–776. doi: 10.1126/science.1252723

Seidel, K., Poeggel, G., Holetschka, R., Helmeke, C., and Braun, K. (2011). Paternal deprivation affects the development of corticotrophin-releasing factor-expressing neurones in prefrontal cortex, amygdala and hippocampus of the biparental Octodon degus. *J. Neuroendocrinol.* 23, 1166–1176. doi: 10.1111/j.1365-2826.2011.02208.x

Snyder, J. S., Choe, J. S., Clifford, M. A., Jeurling, S. I., Hurley, P., Brown, A., et al. (2009). Adult-born hippocampal neurons are more numerous, faster maturing and more involved in behavior in rats than in mice. *J. Neurosci.* 29, 14484–14495. doi: 10.1523/JNEUROSCI.1768-09.2009

Steinman, M. Q., and Trainor, B. C. (2017). Sex differences in the effects of social defeat on brain and behavior in the California mouse: insights from a monogamous rodent. *Semin. Cell Dev. Biol.* 61, 92–98. doi: 10.1016/j.semcdb.2016.06.021

Tabbaa, M., Lei, K., Liu, Y., and Wang, Z. (2017). Paternal deprivation affects social behaviors and neurochemical systems in the offspring of socially monogamous prairie voles. *Neuroscience* 343, 284–297. doi: 10.1016/j.neuroscience.2016.12.011

Tyrka, A. R., Wier, L., Price, L. H., Ross, N., Anderson, G. M., Wilkinson, C. W., et al. (2008). Childhood parental loss and adult hypothalamic-pituitary-adrenal function. *Biol. Psychiatry* 63, 1147–1154. doi: 10.1016/j.biopsych.2008.01.011

Viviani, B., Boraso, M., Valero, M., Gardoni, F., Marco, E. M., Llorente, R., et al. (2014). Early maternal deprivation immunologically primes hippocampal synapses by redistributing interleukin-1 receptor type I in a sex dependent manner. *Brain. Behav. Immun.* 35, 135–143. doi: 10.1016/j.bbi.2013.09.008

Walker, E. A., Foley, J. J., Clark-Vetri, R., and Raffa, R. B. (2011). Effects of repeated administration of chemotherapeutic agents tamoxifen, methotrexate, and 5-fluorouracil on the acquisition and retention of a learned response in mice. *Psychopharmacology* 217, 539–548. doi: 10.1007/s00213-011-2310-8

Wang, J., Fang, Q., and Yang, C. (2017). Effects of paternal deprivation on cocaine-induced behavioral response and hypothalamic oxytocin immunoreactivity and serum oxytocin level in female mandarin voles. *Behav. Brain Res.* 334, 135–141. doi: 10.1016/j.bbr.2017.07.036

Wang, J., Tai, F., Yan, X., and Yu, P. (2012). Paternal deprivation alters play-fighting, serum corticosterone and the expression of hypothalamic vasopressin and oxytocin in juvenile male mandarin voles. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 198, 787–796. doi: 10.1007/s00359-012-0748-8

Wang, L., Zhang, W., Wu, R., Kong, L., Feng, W., Cao, Y., et al. (2014). Neuroendocrine responses to social isolation and paternal deprivation at different postnatal ages in Mandarin voles. *Dev. Psychobiol.* 56, 1214–1228. doi: 10.1002/dev.21202

West, M. J., Slomianka, L., and Gundersen, H. J. (1991). Unbiased stereological estimation of the total number of neurons in thesubdivisions of the rat hippocampus using the optical fractionator. *Anat. Rec.* 231, 482–497. doi: 10.1002/ar.1092310411

Wright, S. L., and Brown, R. E. (2002). The importance of paternal care on pup survival and pup growth in *Peromyscus californicus* when required to work for food. *Behav. Processes* 60, 41–52. doi: 10.1016/s0376-6357(02)00101-8

Wu, M. V., Sahay, A., Duman, R. S., and Hen, R. (2015). Functional differentiation of adult-born neurons along the septotemporal axis of the dentate gyrus. *Cold Spring Harb. Perspect. Biol.* 7:a018978. doi: 10.1101/cshperspect.a018978

Wu, R., Song, Z., Wang, S., Shui, L., Tai, F., Qiao, X., et al. (2014). Early paternal deprivation alters levels of hippocampal brain-derived neurotrophic factor and glucocorticoid receptor and serum corticosterone and adrenocorticotropin in a sex-specific way in socially monogamous mandarin voles. *Neuroendocrinology* 100, 119–128. doi: 10.1159/000366441

Wynne-Edwards, K. E. (1987). Evidence for obligate monogamy in the Djungarian hamster, *Phodopus campbelli*: pup survival under different parenting conditions. *Behav. Ecol. Sociobiol.* 20, 427–437. doi: 10.1007/bf00302986

Wynne-Edwards, K. E., and Lisk, R. D. (1989). Differential effects of paternal presence on pup survival in two species of dwarf hamster (*Phodopus sungorus* and *Phodopus campbelli*). *Physiol. Behav.* 45, 465–469. doi: 10.1016/0031-9384(89)90059-0

Yun, S., Donovan, M. H., Ross, M. N., Richardson, D. R., Reister, R., Farnbauch, L. A., et al. (2016). Stress-induced anxiety- and depressive-like phenotype associated with transient reduction in neurogenesis in adult nestin-CreERT2/diphtheria toxin fragment A transgenic mice. *PLoS One* 11:e0147256. doi: 10.1371/journal.pone.0147256

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer FRD and handling Editor declared their shared affiliation.

frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
published: 24 August 2018
doi: 10.3389/fnbeh.2018.00192

Adolescent Changes in Cellular Proliferation in the Dentate Gyrus of Male and Female C57BL/6N Mice Are Resilient to Chronic Oral Corticosterone Treatments

*Ashna Shome, Razia Sultana, Alina Siddiqui and Russell D. Romeo**

Department of Psychology and Neuroscience and Behavior Program, Barnard College of Columbia University, New York City, NY, United States

Adolescent development is marked by significant changes in neurobiological structure and function. One such change is the substantial adolescent-related decline in cellular proliferation and neurogenesis in the dentate gyrus of the hippocampal formation. Though the behavioral implications of these developmental shifts in cell proliferation are unclear, these changes might contribute to the altered cognitive and emotional functions associated with puberty and adolescence. The significant decrease in cellular proliferation throughout adolescence might make the hippocampus more vulnerable to perturbations during this developmental stage, particularly to factors known to disrupt neurogenesis, such as chronic exposure to stress-related hormones. To examine this possibility, we first measured cellular proliferation in the dentate gyrus of male and female C57BL/6N mice before and after adolescence and then assessed both cellular proliferation and the number of immature neurons in mice treated with oral corticosterone for 4 weeks during either adolescence or adulthood. We found significant age-related decreases in hippocampal cellular proliferation in both males and females. Though the greatest decrease in proliferation was during adolescence, we also observed that proliferation continued to decline through young adulthood. Despite the significant effect of chronic oral corticosterone on body weight gain in both the adolescent- and adult-treated males and females and the subtle, but significant suppressive effect of corticosterone on the number of immature neurons in the adolescent-treated males, cell proliferation in the hippocampus was unaffected by these treatments. These data show that the substantial adolescent-related change in cellular proliferation in the dentate gyrus is largely unaffected by chronic oral corticosterone exposure in males and females. Thus, despite being vulnerable to the metabolic effects of these chronic corticosterone treatments, these results indicate that the developmental changes in cellular proliferation in the dentate gyrus are relatively resilient to these treatments in mice.

Keywords: adolescence, corticosterone, puberty, oral administration, proliferation, neurogenesis, hippocampus

OPEN ACCESS

Edited by:
Gretchen N. Neigh, Virginia Commonwealth University, United States

Reviewed by:
Deborah Suchecki, Federal University of São Paulo, Brazil
Jean-Philippe Guilloux, Université Paris-Sud, France

****Correspondence:***
Russell D. Romeo
rromeo@barnard.edu

Received: *22 May 2018*
Accepted: *07 August 2018*
Published: *24 August 2018*

Citation:
Shome A, Sultana R, Siddiqui A and Romeo RD (2018) Adolescent Changes in Cellular Proliferation in the Dentate Gyrus of Male and Female C57BL/6N Mice Are Resilient to Chronic Oral Corticosterone Treatments. Front. Behav. Neurosci. 12:192. doi: 10.3389/fnbeh.2018.00192

INTRODUCTION

Adolescence is associated with significant neurobiological changes, including substantial declines in cellular proliferation and neurogenesis in the dentate gyrus of the hippocampal formation in both rats and mice (Heine et al., 2004; Kim et al., 2004; He and Crews, 2007; Hodes et al., 2009; Ho et al., 2012). Though the hippocampal formation plays a role in many cognitive and emotional processes (Fanselow and Dong, 2010), the neurobehavioral implications of these adolescent changes in proliferation are unclear. In addition to development, exposure to stress and stress-related hormones, such as corticosterone, alter cell proliferation and neurogenesis in the dentate gyrus (Schoenfeld and Gould, 2012; Opendak and Gould, 2015). Given the marked increase in stress-related physiological and behavioral dysfunctions associated with adolescence, ranging from obesity to mood disorders (Turner and Lloyd, 2004; Dahl and Gunnar, 2009; Lee et al., 2014; Poyrazoglu et al., 2014), perturbations of adolescent hippocampal development by stress-related hormones might contribute to the change in these vulnerabilities.

We have recently shown that chronically exposing adolescent and adult male mice to oral corticosterone leads to significant changes in body weight and adiposity at both ages, but the trajectory and magnitude of these metabolic changes are different before and after adolescence (Kinlein et al., 2017). In particular, despite similarly elevated circulating levels of corticosterone achieved by these treatments, oral corticosterone during adolescence results in initial reduced weight gain followed by increases in body weight, while in adulthood these treatments lead to more linear and substantial increases in both body weight and adiposity (Kinlein et al., 2017). The impact of chronic oral corticosterone treatments on hippocampal cellular proliferation and neurogenesis during adolescence is currently unknown, but may also show age-dependent effects like those observed in the context of metabolism. As alluded to above, it has been shown that exposing adult rats and mice to chronically elevated corticosterone levels reduce hippocampal proliferation and neurogenesis (Murray et al., 2008; David et al., 2009; Brummelte and Galea, 2010; Rainer et al., 2012; Kott et al., 2016). Thus, given the substantial developmental change in proliferation and neurogenesis and the ability of corticosterone to disrupt these processes, it is possible that the chronic oral corticosterone treatments known to affect metabolism differentially during adolescence and adulthood will also result in age-dependent perturbations of these neurobiological parameters.

The purpose of the present set of experiments was to further explore adolescent-related changes in hippocampal proliferation and determine the effects of chronic oral corticosterone on hippocampal proliferation and number of immature neurons in both adolescent and adult male and female mice. Specifically, in the first set of experiments, we examined changes in hippocampal cellular proliferation in male and female mice before and after adolescence, as well during young adulthood. Based on studies in male mice (He and Crews, 2007), we hypothesized that female mice would also show adolescent-related decreases in hippocampal proliferation. In the second set of experiments, we exposed male and female mice to oral corticosterone treatments during either adolescence or adulthood. Given the greater change in hippocampal cellular proliferation and neurogenesis during adolescence (Heine et al., 2004; Kim et al., 2004; He and Crews, 2007; Hodes et al., 2009; Ho et al., 2012), the age-dependent sensitivity to oral corticosterone in the context of metabolism, and the effects of corticosterone on these parameters in adulthood (Murray et al., 2008; David et al., 2009; Brummelte and Galea, 2010; Rainer et al., 2012; Kott et al., 2016), we hypothesized that corticosterone treatment would lead to different effects in the adolescent- compared to adult-treated mice. Finally, though we did not compare males and females directly, the inclusion of both sexes in these studies allowed us to explore whether males and females are affected differently by these treatments, as previous studies report sex differences in the response of the hippocampus to stress-related hormones (Gobinath et al., 2015).

MATERIALS AND METHODS

Animals and Housing

Male and female C57BL/6N mice were obtained from Charles River Laboratories (Wilmington, MA, USA) and allowed to acclimate for at least 1 week prior to the start of the experiments. Mice were housed in pairs (same sex and age) in polycarbonate cages (28 × 17 × 12 cm) with bed-o'cobs 1/4 inch bedding and maintained on a 12-h light dark schedule (lights on at 8:00 h). The temperature was maintained at 21 ± 2°C and mice had *ad libitum* access to water and rodent chow (Lab Diet #5012; PMI Nutrition International LLC, Brentwood, MO, USA). The stage of the estrous cycle was not determined in the female mice. All procedures were approved by the Institutional Animal Care and Use Committee of Columbia University.

Experimental Designs and Tissue Collections

Four experiments were conducted (**Figure 1A**). In the first two experiments, untreated male (Experiment 1) and female (Experiment 2) mice were weighed and perfused (see below) at 30, 58, 70, or 98 days of age (d) and brains were collected (n = 4–6 per age). Though the exact age span that defines adolescence and young adulthood in mice is unclear, these ages are operationally defined in these experiments as pre-adolescent (30 days), post-adolescent (58 days), young adult (70 days) and adult (98 days). In the second two experiments, pre-adolescent (30 days) and young adult (70 days) males (Experiment 3) and females (Experiment 4) were exposed to one of two treatments: 0 or 100 μg/ml corticosterone (C2505; Sigma-Aldrich, St. Louis, MO, USA) in a 1% ethanol and tap water vehicle (n = 6–8 per age and dose). The dose and vehicle used in these studies were based on previously published experiments in adolescent and adult mice (Kinlein et al., 2017). As corticosterone is hydrophobic, it was first dissolved in 100% ethanol via sonication and then added to tap water to a 1% concentration. Animals were exposed

to these treatments for 4 weeks, and therefore, ended at either 58 days or 98 days. Treatments were terminated ∼1 h prior to tissue collection. A pilot study revealed that exposing adolescent or adult mice to the 1% ethanol vehicle for 4 weeks did not significantly affect the number of proliferating cells or immature neurons in the dentate gyrus, and thus, to reduce animal numbers, a tap water only control group was not included in these experiments (Romeo, unpublished observation). Moreover, as tissues from males and females were collected from different sex-specific cohorts of animals and processed at different times, results from each sex were analyzed separately.

For all experiments, animals were perfused after being weighed and administered an overdose of ketamine (80 mg/kg, i.p.p) and xylazine (5 mg/kg, i.p.). Transcardial perfusions were conducted using heparinized saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Brains were removed and post-fixed in 4% paraformaldehyde for 24 h and then incubated in 20% sucrose in 0.1 M PB for 24 h. Brains were snap frozen on powdered dry ice and stored at −80°C until they were sectioned at 35 μm on a coronal plane. The sections were stored in cryoprotectant (1:1 of 20% sucrose in 0.1 M PB and ethylene glycol) at −20°C until immunohistochemistry was performed.

Immunohistochemistry

For all the experiments, 3–4 anatomically matched sections through the dorsal hippocampus separated by 105 μm (corresponding to plates 43–47 in a standard mouse atlas; Franklin and Paxinos, 2008), were processed for either Ki-67 to measure cellular proliferation or doublecortin (DCX) to measure the number of immature neurons. The sections were washed in 0.1 M PB followed by a 5 min incubation in 0.3% H_2O_2 and washed with 0.1 M PB with 0.1% Triton-X-100 (PBT). Sections were then incubated for 1 h in 2% normal goat serum (NGS), and then in either rabbit anti-Ki-67 (1:8,000; AB15580; Abcam, Cambridge, MA, USA) or guinea pig anti-DCX (1:10,000, AB2253; Millipore Sigma, Burlington, MA, USA) for 24 h at 4°C. Sections were then washed in PBT and incubated in goat anti-rabbit or goat anti-guinea pig secondary (1:200; Vector Laboratories, Burlingame, CA, USA) and then exposed to Avidin-Biotin Complex (1:250; Vectastain ABC Kit, Vector Laboratories) for 1 h at room temperature. The tissue was then washed in 0.1 M phosphate buffer saline (PBS) and exposed to 3,3′diaminobenzidine (DAB) in a 3 M sodium acetate buffer containing 0.05% H_2O_2 for 5 min followed by washes in PBS. For Ki-67, the DAB was nickel-enhanced. The tissue was mounted on Fisher Brand Plus slides (Fischer Scientific, Pittsburg, PA, USA) dried and exposed to 70%, 95% and 100% ethanol, followed by xylenes, and cover slipped with DPX (06552, Sigma-Aldrich). Tissue processed for Ki-67 was counter-stained with cresyl violet (C1791, Sigma-Aldrich) prior to coverslipping to measure the cross-sectional area of the dentate gyrus (see below).

Microscopy and Histological Quantification

Ki-67-positive cells in both the upper and lower blades of the dentate gyrus were counted using a light microscope with a 10× objective (Zeiss 200 M Axiovert), while the cross-sectional area of the dentate gyrus was analyzed with ImageJ from pictures taken under a 2.5× objective (**Figure 1B**). Bilateral assessments were made from each section and number of cells and cross-sectional areas were averaged. Based on the cross-sectional measurements, Ki-67-positive cells are expressed as the average number of cells per 100 μm^2 of dentate gyrus. **Figure 1B** provides a representative photomicrograph of the Ki-67 cell counts and cross-sectional area of the dentate gyrus.

DCX-positive cell counts were made under a 40× objective in both the upper and lower blades of the dentate gyrus by placing a grid of 10,000 μm^2 superimposed on top of the images (**Figure 1C**). Bilateral counts from each stained section were averaged and data are expressed as the average number of DCX-positive cells per 10,000 μm^2. **Figure 1C** provides a representative photomicrograph of DCX-positive cells and the approximate placement of the grid used for analysis.

Statistical Analyses

Prior to statistical analyses, the normality of the data sets was confirmed with Shapiro-Wilk normality tests. In Experiments 1 and 2, one-way ANOVAs were used to analyze differences in body weights and the number of Ki-67-positive cells and cross-sectional areas of the dentate gyrus at 30 days, 58 days, 70 days and 98 days. In Experiments 3 and 4, two-way ANOVAs (age of exposure × treatment condition) were used to analyze the number of Ki-67- and DCX-positive cells and cross-sectional area of the dentate gyrus in response to either 0 or 100 μg/ml of oral corticosterone exposure during 4 weeks of either adolescence or adulthood. Significant main effects and interactions were further analyzed with Tukey's honestly significant difference *post hoc* tests. Data are reported as the mean ± SEM and differences were considered significant at $p < 0.05$. All statistical analyses were performed using GraphPad PRISM, version 7.04 (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

Experiments 1 and 2: Developmental Changes in Hippocampal Cellular Proliferation

Experiment 1

In males, body weight increased significantly during adolescence and young adulthood ($F_{(3,20)} = 69.02$, $P < 0.05$), such that 98-day males weighed the most while the 30-day males weighed the least (**Table 1**). In the hippocampus, though there was no significant effect of age on the cross-sectional area of the dentate gyrus ($P = 0.24$; **Figure 2A**), the number of Ki-67-positive cells per 100 μm^2 of dentate gyrus decreased significantly during adolescence ($F_{(3,20)} = 28.50$, $P < 0.05$). Specifically, 30-day males had significantly greater numbers of proliferating cells than 58-day, 70-day, or 98-day males (**Figure 2B**). Though not statistically significant, there appears

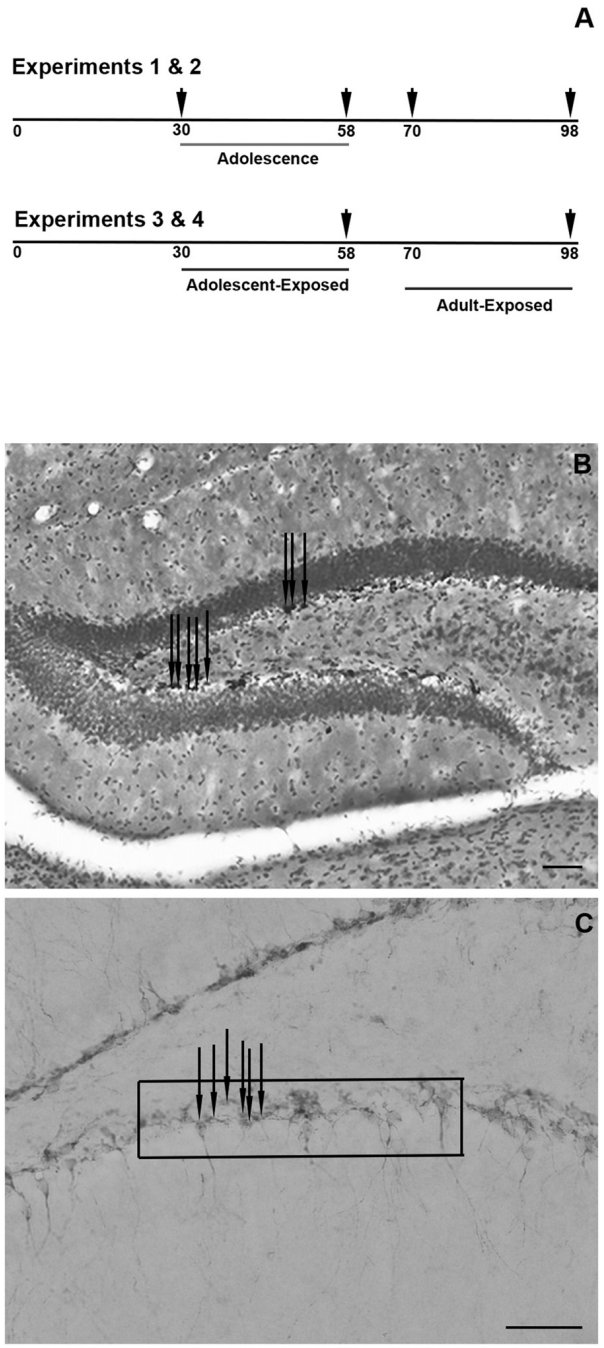

FIGURE 1 | Schematic timeline of the experiments, with arrowheads indicating the times of tissue collections **(A)**. Representative photomicrograph of the counter-stained dentate gyrus and Ki-67-positive cells **(B)** and representative photomicrograph of the doublecortin (DCX)-positive cells in the dentate gyrus and the approximate placement of the grid used to quantify the cells **(C)** Scale bars in panels **(B,C)** = 50 μm. Note that the arrows in panels **(B,C)** are indicating a few examples of Ki-67- and DCX-positive cells, respectively.

to be a trend toward a continued decrease in the number of Ki-67-positive cells through 58-day, 70-day and 98-day animals.

TABLE 1 | Mean (±SEM) body weight of male and female mice in Experiments 1 (male mice) and 2 (female mice).

Sex and days of age (d)	Body weight (g)
Male 30d	17.1 ± 0.2^{a}
Male 58d	24.1 ± 0.7^{b}
Male 70d	24.9 ± 0.5^{b}
Male 98d	28.2 ± 0.5^{c}
Female 30d	15.7 ± 0.7^{a}
Female 58d	19.1 ± 0.6^{b}
Female 70d	21.8 ± 0.4^{c}
Female 98d	24.1 ± 0.5^{d}

Numbers that share a letter within an experiment are not significantly different from one another ($P < 0.05$).

Experiment 2

In females, there was also a significant change in body weight throughout adolescence and young adulthood ($F_{(3,16)} = 38.92$, $P < 0.05$), such that body weight significantly increased in a linear manner at all four ages measured (**Table 1**). For the measurements made in the hippocampus, there were both significant increases in the cross-sectional area of the dentate gyrus and decreases in the number of Ki-67-positive cells across the ages ($F_{(3,16)} = 4.52$ and 23.52. respectively, $P < 0.05$). For cross-sectional area, the dentate gyrus was significantly larger in 98-day females compared to 30-day, 58-day, or 70-day females (**Figure 2C**), while the Ki-67 cells per 100 μm^2 of dentate gyrus show the same adolescent-related decline as males, with 30-day females having the greatest number of Ki-67 cells compared to all the other ages (**Figure 2D**). Also similar to the males, it appears Ki-67 cell number continues to decline in the dentate gyrus through late adolescence and young adulthood, with the lowest number of cells in the 98-day females.

Experiments 3 and 4: Hippocampal Cellular Proliferation and Number of Immature Neurons Following Oral Corticosterone Treatment During Adolescence or Adulthood

Experiment 3

For body weight in the adolescent- and adult-treatment males, main effects were found, such that adult-treated males weighed more than adolescent-treated males, and animals treated with 100 μg/ml of corticosterone were heavier than the animals treated with 0 μg/ml of corticosterone ($F_{(1,28)} = 75.35$ and 41.12, respectively, $P < 0.05$; **Table 2**). For the dependent variables measured in the dentate gyrus, we found no main effects or interaction of age of exposure or treatment condition on the cross-sectional area (**Figure 3A**), and only a significant main effect of age on the number of Ki-67 cells per 100 μm^2 of the dentate gyrus ($F_{(1,24)} = 7.17$, $P < 0.05$). Specifically, the 58-day animals treated with either 0 or 100 μg/ml of corticosterone during adolescence had a greater number of Ki-67 cells than the 98-day animals treated with either 0 or 100 μg/ml of corticosterone during adulthood (**Figure 3B**). There was no main effect of corticosterone treatment or interaction between age of exposure

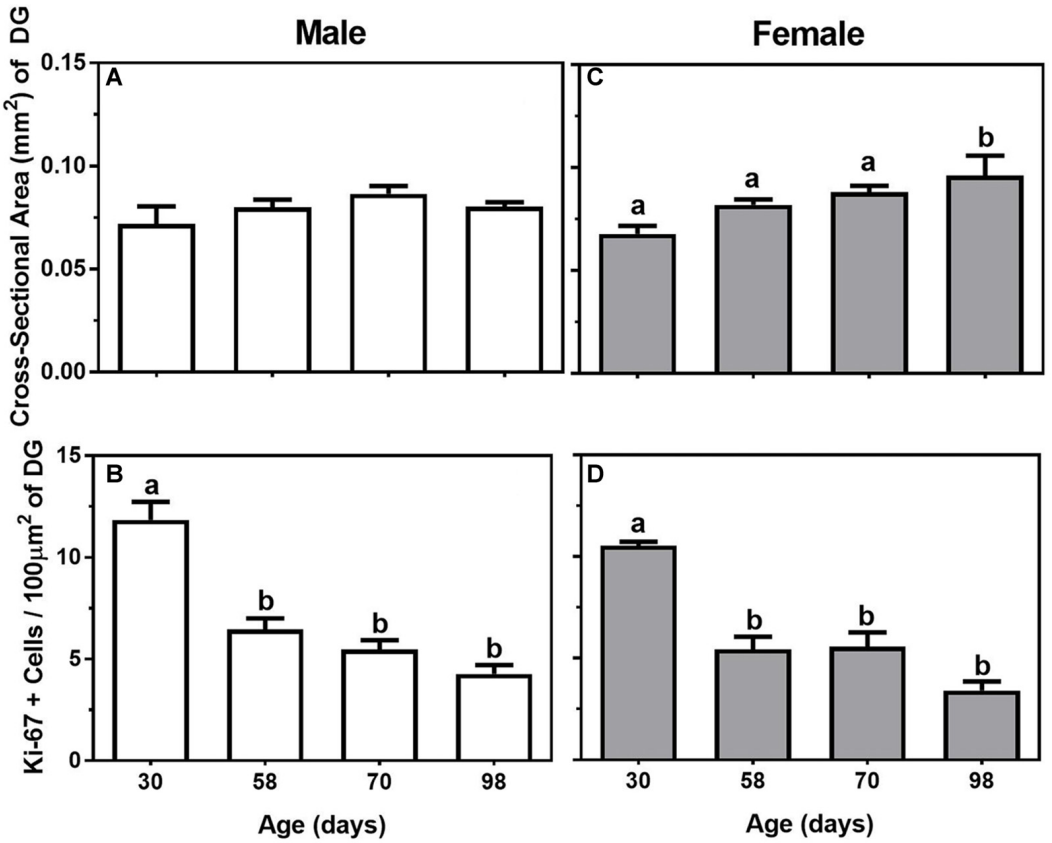

FIGURE 2 | Mean (±SEM) cross-sectional area (mm^2) of the dentate gyrus (DG) and number of Ki-67-positive cells per 100 μm^2 of DG in 30-, 58-, 70-, and 98-day-old male (**A,B**; left panels) and female (**C,D**; right panels) mice. Bars that share a letter are not significantly different from one another.

and corticosterone treatment on Ki-67 cells number per 100 μm^2 of dentate gyrus.

For the number of DCX-positive cells in the dentate gyrus, we found both a significant main effect of age of exposure as well as a significant interaction between age of exposure and corticosterone treatment ($F_{(1,24)}$ = 36.35 and 4.35, respectively, $P < 0.05$). Specifically, like the number of Ki-67-positive cells, the number of DCX cells were greater in the 58-day compared to 98-day males, independent of treatment. For the interaction, we found a slight, but significant suppressive effect of corticosterone on DCX cell number, but only when the exposure occurred during adolescence (**Figure 3C**). There was no significant main effect of treatment condition on the number of DCX cells in the dentate gyrus of males.

Experiment 4

Similar to males, main effects were found on the body weights of the adolescent- and adult-treatment females, such that adult-treated females weighed more than adolescent-treated females, and females treated with 100 μg/ml of corticosterone were heavier than the females treated with 0 μg/ml of corticosterone ($F_{(1,26)}$ = 14.16 and 25.28, respectively, $P < 0.05$; **Table 2**). Also similar to the males, we found no main effects or interaction of age of exposure or treatment condition on the cross-sectional area of the dentate gyrus (**Figure 4A**), but found a significant main effect of age on the number of Ki-67 cells per 100 μm^2 of the dentate gyrus ($F_{(1,22)}$ = 6.03, $P < 0.05$). Like the males, 58-day females treated with either 0 or 100 μg/ml of corticosterone during adolescence had a greater number of Ki-67 cells than 98-day females treated with either 0 or 100 μg/ml

TABLE 2 | Mean (±SEM) body weight of male and female mice in Experiments 3 (male mice) and 4 (female mice) treated with either 0 or 100 μg/ml of corticosterone (CORT) during either adolescence (30–58 days) or young adulthood (70–98 days).

Sex and age of exposure	Treatment condition	Body weight (g)
Male Adolescent-Exposed	0 μg/ml CORT	23.6 ± 0.5
Male Adolescent-Exposed	100 μg/ml CORT	26.3 ± 0.8*
Male Adult-Exposed	0 μg/ml CORT	27.7 ± 0.5
Male Adult-Exposed	100 μg/ml CORT	32.8 ± 0.5*
Female Adolescent-Exposed	0 μg/ml CORT	20.6 ± 0.4
Female Adolescent-Exposed	100 μg/ml CORT	24.4 ± 0.9*
Female Adult-Exposed	0 μg/ml CORT	23.4 ± 0.6
Female Adult-Exposed	100 μg/ml CORT	27.8 ± 1.0*

Asterisks indicate a significant difference between the 0 μg/ml and 100 μg/ml treatment conditions. Note that the significant main effects for age of exposure in Experiments 3 and 4 are not indicated.

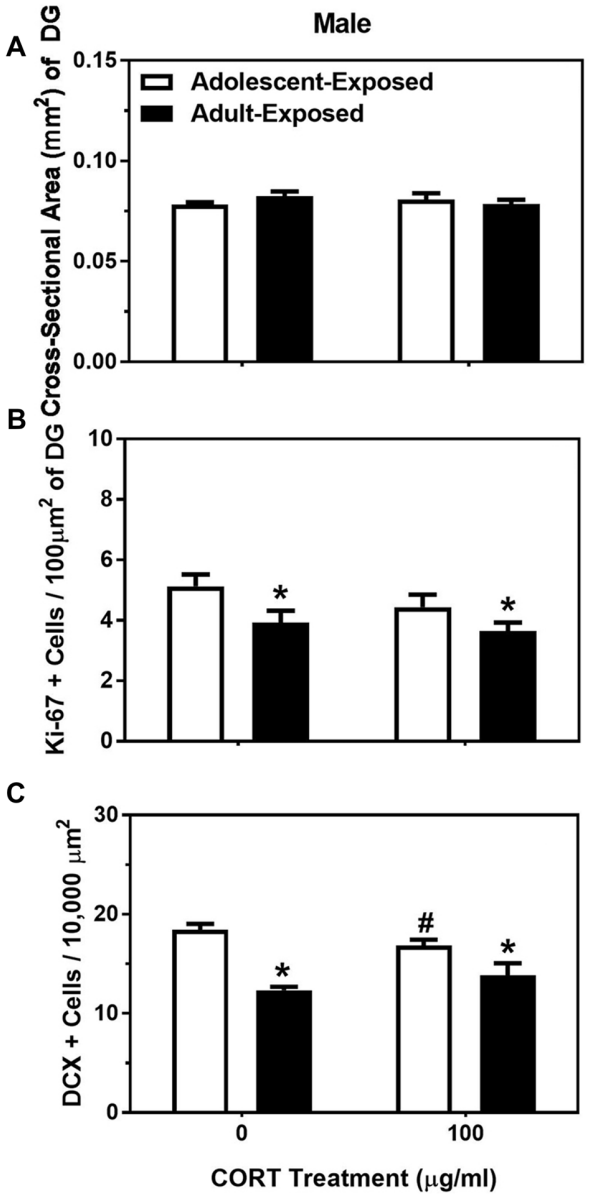

FIGURE 3 | Mean (±SEM) cross-sectional area (mm^2) of the DG **(A)**, number of Ki-67-positive cells per 100 μm^2 of DG **(B)** and number of DCX-positive cells per 10,000 μm^2 **(C)** in male mice exposed to either 0 or 100 μg/ml of corticosterone (CORT) during adolescence (white bars) or adulthood (black bars). Asterisks indicate a significant difference between the adolescent-exposed and adult-exposed animals, while #indicates a significant difference between the 0 μg/ml and 100 μg/ml dose of CORT in the adolescent-exposed animals.

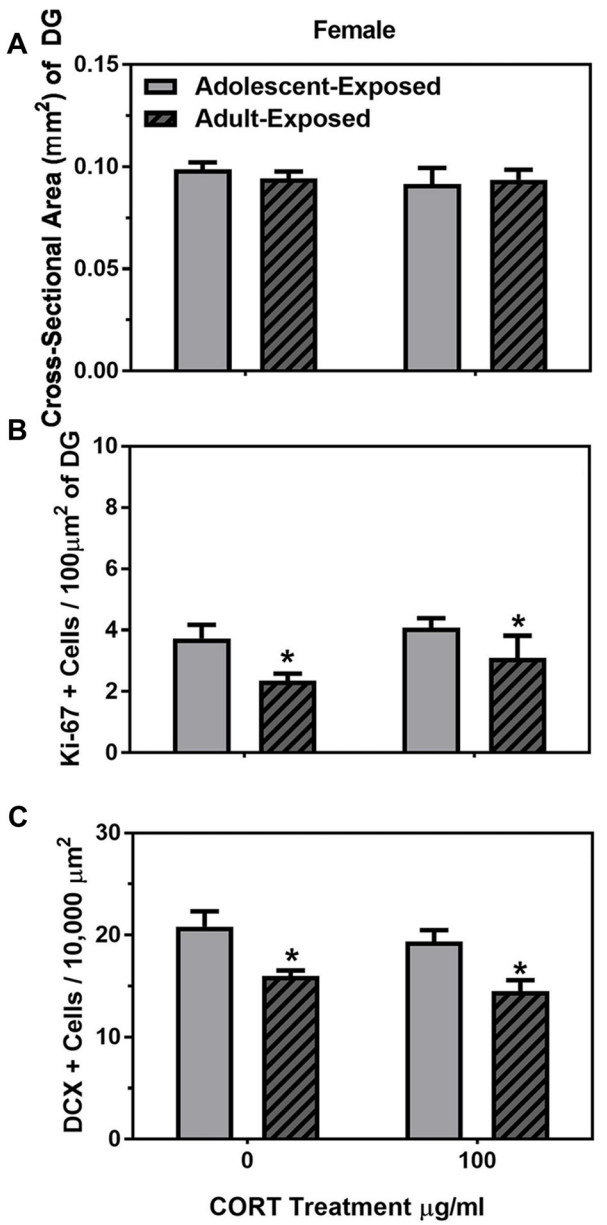

FIGURE 4 | Mean (±SEM) cross-sectional area (mm^2) of the DG **(A)** number of Ki-67-positive cells per 100 μm^2 of DG **(B)** and number of DCX-positive cells per 10,000 μm^2 **(C)** in female mice exposed to either 0 or 100 μg/ml of CORT during adolescence (light gray bars) or adulthood (dark gray bars with hash marks). Asterisks indicate a significant difference between the adolescent-exposed and adult-exposed animals.

of corticosterone during adulthood (**Figure 4B**). There was no main effect of corticosterone treatment or interaction between age of exposure and corticosterone treatment in the females.

For DCX cells counts, we found only a significant main effect of age of exposure ($F_{(1,22)} = 20.81$, $P < 0.05$), such that 58-day females had a greater number of DCX-positive cells than 98-day females, independent of treatment condition (**Figure 4C**). There was no main effect of treatment condition or interaction between age of exposure and treatment condition on the number of DCX-positive cells in the female dentate gyrus.

DISCUSSION

These data indicate that cellular proliferation in the dentate gyrus showed significant declines during adolescent development in

both male and female C57BL/6N mice. Furthermore, despite significant somatic changes in response to these corticosterone treatments, we found little effect of these treatments on hippocampal proliferation and the number of immature neurons. Specifically, chronic corticosterone exposure had no effect on these parameters in adolescent- or adult-treated females, and in males, only the number of immature neurons was affected when these treatments occurred during adolescence. Thus, counter to the original hypothesis, these data indicate that the substantial change in hippocampal proliferation and neurogenesis that occurs during adolescence is largely resistant to these chronic oral corticosterone treatments.

The metabolic function of the animals was influenced by this exposure to corticosterone, as has been reported previously (Karatsoreos et al., 2010; Cassano et al., 2012; Kinlein et al., 2017). That is, these corticosterone treatments led to significant weight gain in the adolescent- and adult-exposed subjects. Thus, the relative lack of corticosterone-induced changes in cellular proliferation and number of immature neurons indicates a dissociation between the effects of corticosterone on somatic and neurobiological functions. It is possible that a higher dose of corticosterone would have yielded a greater effect on the neurobiological parameters we assessed, as the slight decrease in DCX cell number in the adolescent-treated males suggests that the 100 μg/ml dose of corticosterone might be near an effective threshold. Furthermore, a longer time of exposure might be needed, as others have reported that 7 weeks of oral corticosterone exposure can reduce hippocampal proliferation in adult male mice (David et al., 2009). Regardless, if this method of delivery is to be used to understand the influence of corticosterone on either hippocampal cellular proliferation or neurogenesis in either adolescent or adult mice, then additional experiments will be needed to address these dose response and time course issues.

Given the relative absence of an effect of corticosterone on hippocampal proliferation in the present study, these data however do raise an interesting possibility that the metabolic changes induced by these treatments might have protected the dentate gyrus from any adverse effects of chronic corticosterone exposure. For example, previous research has indicated that metabolic hormones, such as leptin, can be neuroprotective (Avraham et al., 2011), and leptin has been shown to reverse the suppressive effects of chronic unpredictable stress on hippocampal neurogenesis in rats (Garza et al., 2012). Leptin levels have been reported to be increased in mice treated with the dose of oral corticosterone used in the present study (Karatsoreos et al., 2010). Moreover, the dentate gyrus has a relatively high expression level of leptin receptors in C57BL/6 mice (Huang et al., 1996). Thus, it is possible that the elevated leptin levels induced by these treatments might have mitigated the suppressive effects of corticosterone on hippocampal cellular proliferation. Future experiments will be needed to address this possibility.

We found that, independent of corticosterone treatment, cell proliferation and the number of immature neurons were significantly different between 58 days and 98 days of age in both females and males, indicating that these parameters of plasticity are not static during adulthood, but continue to decrease. While previous work has observed a substantial decrease in hippocampal cellular proliferation and neurogenesis between adolescent and adult mice (He and Crews, 2007), differences have not been previously measured at different ages during young adulthood in mice. Given the important role of hippocampal proliferation in neurobehavioral functions, ranging from learning and memory to emotionality (Bannerman et al., 2014), future studies will need to probe the functional implications of these changes in the dentate gyrus during both adolescence and young adulthood.

Taken together, these data indicate that despite profound changes in hippocampal cellular proliferation and neurogenesis during adolescence and adulthood, chronic oral corticosterone exposure was largely unable to disrupt this developmental process in male or female mice. Though oral corticosterone may serve as a useful model to understand both adolescent- and adult-related differences in metabolic dysfunctions (Kinlein et al., 2017), the present data suggest this method may not be an effective way to examine the role of corticosterone on hippocampal neurogenesis in mice. Instead, we propose that this methodology may be appropriate for future studies trying to understand the interaction between metabolic dysregulation and neurobiological functions, and the potential compensatory mechanisms that metabolic hormones may have on deleterious effects of chronic exposure to stress or stress-related hormones on the brain and behavior.

AUTHOR CONTRIBUTIONS

AShome and RR designed and conducted the experiments, while RS and ASiddiqui helped conduct the experiments. All authors contributed to the writing and editing of the manuscript.

FUNDING

This work was supported in part by a Faculty Research Grant from Barnard College.

ACKNOWLEDGMENTS

We would like to thank Page Buchanan for excellent animal care.

REFERENCES

Avraham, Y., Davidi, N., Lassri, V., Vorobiev, L., Kabesa, M., Dayan, M., et al. (2011). Leptin induces neuroprotection neurogenesis and angiogenesis after stroke. *Curr. Neurovasc. Res.* 8, 313–322. doi: 10.2174/156720211798120954

Bannerman, D. M., Sprengel, R., Sanderson, D. J., McHugh, S. B., Rawlins, J. N. P., Monyer, H., et al. (2014). Hippocampal synaptic plasticity spatial memory and anxiety. *Nat. Rev. Neurosci.* 15, 181–192. doi: 10.1038/nrn3677

Brummelte, S., and Galea, L. A. M. (2010). Chronic high corticosterone reduces neurogenesis in the dentate gyrus of adult male and female rats. *Neuroscience* 168, 680–690. doi: 10.1016/j.neuroscience.2010.04.023

Cassano, A. E., White, J. R., Penraat, K. A., Wilson, C. D., Rasmussen, S., and Karatsoreos, I. N. (2012). Anatomic, hematologic, and biochemical features of C57BL/6NCrl mice maintained on chronic oral corticosterone. *Comp. Med.* 62, 348–360.

Dahl, R. E., and Gunnar, M. R. (2009). Heightened stress responsiveness and emotional reactivity during pubertal maturation: implications for psychopathology. *Dev. Psychopathol.* 21, 1–6. doi: 10.1017/s0954579409000017

David, D. J., Samuels, B. A., Rainer, Q., Wang, J. W., Marsteller, D., Mendez, I., et al. (2009). Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron* 62, 479–493. doi: 10.1016/j.neuron.2009.04.017

Fanselow, M. S., and Dong, H.-W. (2010). Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron* 65, 7–19. doi: 10.1016/j.neuron.2009.11.031

Franklin, K. B. J., and Paxinos, G. (2008). *The Mouse Brain in Stereotaxic Coordinates.* New York, NY: Elsevier.

Garza, J. C., Guo, M., Zhang, W., and Lu, X.-Y. (2012). Leptin restores adult hippocampal neurogenesis in a chronic unpredictable stress model of depression and reverses glucocorticoid-induced inhibition of GSK-3b/b-catenin signaling. *Mol. Psychiatry* 17, 790–808. doi: 10.1038/mp.2011.161

Gobinath, A. R., Mahmoud, R., and Galea, L. A. M. (2015). Influence of sex and stress exposure across the lifespan on endophenotype of depression: focus on behavior, glucocorticoid, and hippocampus. *Front. Neurosci.* 8:420. doi: 10.3389/fnins.2014.00420

He, J., and Crews, F. T. (2007). Neurogenesis decreases during brain maturation from adolescence to adulthood. *Pharmacol. Biochem. Behav.* 86, 327–333. doi: 10.1016/j.pbb.2006.11.003

Heine, V. M., Maslam, S., Joëls, M., and Lucassen, P. J. (2004). Prominent decline of newborn cell proliferation, differentiation, and apoptosis in the aging dentate gyrus, in absence of an age-related hypothalamic-pituitary-adrenal axis activation. *Neurobiol. Aging* 25, 361–375. doi: 10.1016/s0197-4580(03)00090-3

Ho, A., Villacis, A. J., Svirsky, S. E., Foilb, A. R., and Romeo, R. D. (2012). The pubertal-related decline in cellular proliferation and neurogenesis in the dentate gyrus of male rats is independent of the pubertal rise in gonadal hormones. *Dev. Neurobiol.* 72, 743–752. doi: 10.1002/dneu.20987

Hodes, G. E., Yang, L., van Kooy, J., Santollo, J., and Shors, T. J. (2009). Prozac during puberty: distinctive effects on neurogenesis as a function of age and sex. *Neuroscience* 163, 609–617. doi: 10.1016/j.neuroscience.2009.06.057

Huang, X. F., Koutcherov, I., Lin, S., Wang, H. Q., and Storlien, L. (1996). Localization of leptin receptor mRNA expression in mouse brain. *Neuroreport* 7, 2635–2638. doi: 10.1097/00001756-199611040-00045

Karatsoreos, I. N., Bhagat, S. M., Bowles, N. P., Weil, Z. M., Pfaff, D. W., and McEwen, B. S. (2010). Endocrine and physiological changes in response to chronic corticosterone: a potential model of metabolic syndrome in mouse. *Endocrinology* 151, 2117–2127. doi: 10.1210/en.2009-1436

Kim, Y.-P., Kim, H., Shin, M.-S., Chang, H.-K., Jang, M.-H., Shin, M.-C., et al. (2004). Age-dependence of the effect of treadmill exercise on cell proliferation in the dentate gyrus of rats. *Neurosci. Lett.* 355, 152–154. doi: 10.1016/j.neulet.2003.11.005

Kinlein, S. A., Shahanoor, Z., Romeo, R. D., and Karatsoreos, I. N. (2017). Chronic corticosterone treatment during adolscence has significant effects on metabolism and skeletal development in male C57BL6/N mice. *Endocrinology* 158, 2239–2254. doi: 10.1210/en.2017-00208

Kott, J. M., Mooney-Leber, S. M., Shoubah, F. A., and Brummelte, S. (2016). Effectiveness of different corticosterone administration methods to elevate corticosterone seurm levels, induce depressive-like behavior, and affect neurogenesis levels in female rats. *Neuroscience* 312, 201–214. doi: 10.1016/j.neuroscience.2015.11.006

Lee, F. S., Heimer, H., Giedd, J. N., Lein, E. S., Šestan, N., Weinberger, D. R., et al. (2014). Adolescent mental health—opportunity and obligation. *Science* 346, 547–549. doi: 10.1126/science.1260497

Murray, F., Smith, D. W., and Hutson, P. H. (2008). Chronic low dose corticosterone exposure decreased hippocampal cell proliferation, volume and induced anxiety and depression like behaviours in mice. *Eur. J. Pharmacol.* 583, 115–127. doi: 10.1016/j.ejphar.2008.01.014

Opendak, M., and Gould, E. (2015). Adult neurogenesis: a substrate for experience-dependent change. *Trend Cogn. Sci.* 19, 151–161. doi: 10.1016/j.tics.2015.01.001

Poyrazoglu, S., Bas, F., and Darendeliler, F. (2014). Metabolic syndrome in young people. *Curr. Opin. Endocrinol. Diabetes Obes.* 21, 56–63. doi: 10.1097/01.med.0000436414.90240.2c

Rainer, Q., Xia, L., Guilloux, J. P., Gabriel, C., Mocaër, E., Hen, R., et al. (2012). Beneficial behavioural and neurogenic effects of agomelatine in a model of depression/anxiety. *Int. J. Neuropsychopharmacology* 15, 321–335. doi: 10.1017/s1461145711000356

Schoenfeld, T. J., and Gould, E. (2012). Stress, stress hormones, and adult neurogenesis. *Exp. Neurol.* 233, 12–21. doi: 10.1016/j.expneurol.2011.01.008

Turner, R. J., and Lloyd, D. A. (2004). Stress burden and the lifetime incidence of psychiatric disorder in young adults. *Arch. Gen. Psychiatry* 61, 481–488. doi: 10.1001/archpsyc.61.5.481

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
published: 30 August 2018
doi: 10.3389/fnbeh.2018.00188

Asthma Induction During Development and Adult Lung Function, Behavior and Brain Gene Expression

*Jasmine I. Caulfield[1,2,3], Michael J. Caruso[1,3], Rebecca A. Bourne[1], Nicole R. Chirichella[1], Laura C. Klein[1], Timothy Craig[4], Robert H. Bonneau[5], Avery August[6] and Sonia A. Cavigelli[1,2,3]**

[1]Department of Biobehavioral Health, Pennsylvania State University, University Park, PA, United States, [2]The Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, PA, United States, [3]Center for Brain, Behavior, and Cognition, Pennsylvania State University, University Park, PA, United States, [4]Allergy, Asthma & Immunology Section, Departments of Medicine and Pediatrics, Penn State University, Hershey, PA, United States, [5]Departments of Microbiology and Immunology and Pediatrics, Pennsylvania State University College of Medicine, Hershey, PA, United States, [6]Department of Microbiology and Immunology, Cornell University, Ithaca, NY, United States

In developing youth, allergic asthma is the most common chronic condition, with 9%–10% of youth affected. Asthma onset during childhood and adolescence is further associated with other health issues, particularly psychiatric conditions. To understand causal mechanisms by which developmental asthma may lead to altered behavior, brain and health trajectories, we developed a mouse model of developmental allergic asthma. In the current study, we tested for potential long-term effects of developmental asthma on adult lung function and behavior and brain gene expression associated with emotion and stress regulation. We manipulated airway inflammation (AI) and methacholine (MCH)-induced bronchospasm (resulting in labored breathing, LB) in young male and female BALB/cJ mice and measured adult outcomes 3 months after final asthma manipulations. Results indicated that allergen exposure, used to cause AI, and which ended on post-natal day 56 (P56), led to persistent lung AI, mucus buildup and gene expression related to allergic asthma 3 months after final allergen exposure. In addition, at this same age, early allergen exposure led to altered brain gene expression related to stress regulation (prefrontal corticotropin releasing hormone receptor 1, *Crhr1* and hippocampal glucocorticoid receptor, *GR*) and serotonin function (brainstem serotonin transporter, *SERT*). On the other hand, LB events during development led to altered anxiety-related behavior. Importantly, sex and pre-asthma fear-related behavior (ultrasonic vocalization, USV rates) modulated these adult outcomes. Asthma that develops during childhood/adolescence may have long-term impacts on emotion and stress regulation mechanisms, and these influences may be moderated by sex and pre-asthma temperament.

Keywords: asthma, anxiety, inflammation, house dust mite, methacholine, ultrasonic vocalization

OPEN ACCESS

Edited by:
Erica R. Glasper, University of Maryland, College Park, United States

Reviewed by:
Jamie Lynn Sturgill, University of Kentucky, United States
Ben Nephew, Worcester Polytechnic Institute, United States

****Correspondence:***
Sonia A. Cavigelli
sac34@psu.edu

Received: *02 May 2018*
Accepted: *06 August 2018*
Published: *30 August 2018*

Citation:
Caulfield JI, Caruso MJ, Bourne RA, Chirichella NR, Klein LC, Craig T, Bonneau RH, August A and Cavigelli SA (2018) Asthma Induction During Development and Adult Lung Function, Behavior and Brain Gene Expression. Front. Behav. Neurosci. 12:188. doi: 10.3389/fnbeh.2018.00188

Abbreviations: AI, Airway inflammation; AI+LB, Airway inflammation+labored breathing; CON, Control; HDM, House dust mite; IL, Interleukin; LB, Labored breathing; MCH, Methacholine; P, Postnatal day; USV, Ultrasonic vocalization.

INTRODUCTION

Allergic asthma affects 9.5% of children and adolescents in the United States (Akinbami et al., 2012). People with asthma can develop comorbidities with other atopic disorders and other health outcomes (Guerra et al., 2004; Lødrup Carlsen et al., 2014). Importantly, there is a high comorbidity of allergic and internalizing disorders such as anxiety and depression, conditions that are associated with altered stress and immune regulation (Nascimento et al., 2002; Goodwin et al., 2003; Katon et al., 2007; Ross et al., 2007; Buske-Kirschbaum et al., 2008; Tonelli et al., 2009). Interestingly, research suggests that asthma patients are at greater risk for developing these internalizing disorders as early as adolescence (Dudeney et al., 2017).

Serotonin function, in particular transporter function, has been implicated in the pathology of internalizing disorders. For example, serotonin transporter (*SERT*) knockout mice display anxiety-like behavior, and in patients with depression, negative attitudes are correlated with SERT binding potential (Holmes et al., 2003; Meyer, 2007). Interestingly, serotonin also plays a role in allergic responses—its release in the periphery is part of the T-helper type 2 allergic response, and manipulation of peripheral receptors results in improvement of asthma symptoms in murine models (Nau et al., 2015; Shajib and Khan, 2015). In addition, mast cells, which are found in the skin and mucosal tissues as well as in the central nervous system, play an important role in allergic responses and produce molecules like histamine during reactions (Theoharides et al., 2012; Dong et al., 2014). Mast cells are also responsible for producing 20%–40% of the serotonin in the hippocampus, and they produce serotonin in cases of non-allergic asthma and after injury (Nautiyal et al., 2012; Theoharides et al., 2012; Shajib and Khan, 2015). Thus, one mechanism by which allergic asthma may predispose an individual toward internalizing disorders may be by altered serotonin regulation.

Adolescence is an important time for maturation and growth: many changes occur in the body and brain that are critical for normal development of emotion and stress regulation as well as behavior (Spear, 2000; Tirelli et al., 2003; Dahl, 2004; Romeo, 2010, 2015; Sachser et al., 2011; McCormick and Green, 2013). Chronic stressors during this period of growth can have a negative impact on normal development and lead to increased risk of anxiety- or depression-related internalizing disorders (Spear, 2000; Molnar et al., 2001; Barnum et al., 2012; Moretti and Craig, 2013; Dudeney et al., 2017). These adolescent stress effects can also exacerbate adult allergen-induced immune responses and lung hyper-responsiveness, and they can increase midbrain tumor necrosis factor alpha and interleukin (IL)-1 levels following a later immune challenge (Chida et al., 2007; Barnum et al., 2012). The downstream consequences of adolescent stress can be relatively long lasting. For example, chronic adolescent social and non-social stress in male rats and mice can result in anxiety-like effects from 3 weeks to 28 weeks after the end of stress (McCormick et al., 2008; Chaby et al., 2015; Caruso et al., 2017). Chronic adolescent stress can also cause lasting changes in rat hippocampal soma volume 3 weeks after stress completion (Isgor et al., 2004). Thus, adolescence may be a period when organisms are particularly susceptible to long-term effects of stressors, although it is important to note that adolescence may also be a period of stress resilience (Meyer et al., 2016; Sadler and Bailey, 2016).

While evidence suggests that stressors during adolescence predispose an organism toward adult anxiety, it is possible that a predisposition to anxiety prior to adolescence may heighten individual responses to and/or memory of adolescent stressors. In the case of adolescent asthma, an anxious predisposition may exacerbate inflammatory symptoms, resulting in more severe or persistent asthma, and/or more frequent recollection of these symptoms (Richardson et al., 2006). Research indicates that anxiety can be brought on from experiencing a chronic health challenge and associated adverse medical events (Chida et al., 2008). Additionally, parental anxiety can influence a child, putting them at increased risk for developing an anxiety disorder (Whaley et al., 1999). This bi-directional relationship between asthma and internalizing disorders requires further study to elucidate causal directionality and mechanism.

With regard to asthma-internalizing disorder co-morbidity, there are important sex differences to consider. Young males tend to have a higher prevalence of asthma compared to females, but this ratio changes in adolescence and adulthood such that females have higher rates of asthma than males at these older ages (Anderson et al., 1992; Skobeloff et al., 1992; Katon et al., 2007). Other disorders also show distinct sex-specific diagnosis and prevalence patterns. Males tend to show increased rates of behavioral and developmental disorders like attention deficit hyperactivity disorder compared to females, whereas females tend to exhibit higher rates of anxiety, depression and other mood disorders (Andersen and Teicher, 2000; Abikoff et al., 2002; Roza et al., 2003; Holder and Blaustein, 2014). Among adolescents with asthma, females are at greater risk for being diagnosed with anxiety disorders compared to males (Katon et al., 2007; Ross et al., 2007). The effects of adolescent stress can also be worse in females compared to males (Bourke and Neigh, 2011).

In this manuscript, we focus on two features of allergic asthma that may be important in influencing anxiety development. Airway inflammation (AI) is a classic feature of allergic disorders, including asthma, characterized by enhanced T-helper type 2 immune reaction. Allergen-activated T-helper type 2 cells and IL-33 stimulated Type 2 innate lymphoid cells (ILC2) produce cytokines such as IL-4, IL-5 and IL-13 to promote the allergic response and inflammation (Galli et al., 2008; Lloyd, 2010; Salmond et al., 2012; Sjöberg et al., 2017). Certain polymorphisms of IL-33 have also been correlated with increased risk of developing hay fever earlier than 6 years of age (Schröder et al., 2016). Additionally, asthma is characterized by bouts of respiratory dysfunction, bronchoconstriction and labored breathing (LB), which can occur during acute asthma attacks. This state of difficulty breathing that is often associated with decreased oxygen saturation is a significant stressor, and it

has been associated with respiratory failure, changes in muscle activity, and remodeling of the airways (Smith and Hudgel, 1980; Ahmad et al., 2012). Both chronic AI and repeated acute LB experiences during development may alter brain and behavior development in such a way as to predispose an individual toward internalizing disorders.

Recent work has established a mouse model of peri-adolescent asthma using independent manipulation of AI and LB to determine how these developmental symptoms affect later-life behavior and physiology. This prior work showed that chronic exposure to intranasal allergen that began during the first week of life led to significant AI, inflammatory cytokine expression, mucus production and collagen buildup in the lungs within 2–4 weeks, and inhaled methacholine (MCH) treatments during development led to significant acute LB events (Saglani et al., 2009; Caulfield et al., 2017). In addition, in adulthood, 3 weeks after termination of repeated peri-adolescent acute LB events, mice exhibited increased anxiety-like behavior and altered brain gene expression (Caulfield et al., 2017). In addition, lung inflammation persisted 3 weeks after cessation of chronic allergen exposure during development, and inflammation and airway hyper-responsiveness was more pronounced in females than males (Blacquière et al., 2010; Caulfield et al., 2017). A longitudinal study on humans with asthma determined that childhood asthma severity (at 7 years of age) strongly predicted lung function and persistence of symptoms in adulthood (at 50 years of age; Tai et al., 2014a). In the current study, we manipulated the same characteristics of allergic asthma (AI and LB) to determine behavioral and physiological effects of these peri-adolescent asthma symptoms 3 months after exposure ended. We used our previously-established mouse model and measured behavior, AI/mucus and brain/lung gene expression 3 months after allergen exposure ended (Caulfield et al., 2017). To determine if long-term effects of developmental asthma were specific to a certain sex or moderated by pre-asthma fearful disposition, we studied both males and females and quantified neonate fear-associated behavior (ultrasonic vocalization, USV) prior to experimental asthma induction.

MATERIALS AND METHODS

Experimental Groups and Design

The goal of the present study was to determine what changes persist in mouse adult anxiety-related behavior, gene expression, and corticosterone production 3 months after peri-adolescent asthma symptom exposure. The study used male and female BALB/cJ mice in four peri-adolescent asthma conditions: (1) AI; (2) LB; (3) AI+LB; and (4) Similarly-Handled Controls—CON (**Figure 1A**). Animals were bred in three cohorts to reach a minimum of 10 animals per sex per condition (N = 98, 23–41 mice/cohort). To control for litter effects, same-sex pups from each litter were evenly distributed across all conditions, and all experimental manipulations and data collection were conducted for all littermates at the same time. Body weights were measured on P14, 90 and 140 to determine if the above manipulations altered growth trajectories. By P90, males weighed significantly more than females (P90: $F_{(1,82)} = 125.7$, $p < 0.001$; P140: $F_{(1,81)} = 411.6$, $p < 0.001$), but there were no significant effects of AI, LB, or neonatal USV rates on weight at any age (Fs < 2.65, ps > 0.108). This study was carried out in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research. The protocol was approved by the Pennsylvania State University Institutional Animal Care and Use Committee.

Mouse Breeding and Housing

Male and female BALB/cJ breeders were obtained from Jackson Laboratories (Bar Harbor, ME, USA), and mice were bred in the laboratory. To produce litters of sufficient size, sister-pairs were bred with one male to produce "double-litters" (24 double-litters, mean size: 6.5, not culled). Pup identity was tracked by marking them with non-toxic Sharpie® marker until postnatal day (P) 9, at which point all pups were given permanent, unique ear notches. To quantify pre-manipulation fear-related behavior, USVs were measured and coded on P3-P5 (2 min/day) using the "Isolation" method and recording at 65 Hz (Dichter et al., 1996; Brunelli et al., 1997; Branchi et al., 1998; Hahn and Lavooy, 2005; Caulfield et al., 2017). Several inbred strains of mice, including BALB/cJ mice, display peak USV production at postnatal day P3 with USVs in the range of 60–80 kHz, and the amount of calling decreases during the first 2 weeks of life (Bell et al., 1972). In rodents, USVs can predict later-life emotion regulation; mice selectively bred based on pup frequency of USVs develop into distinct high and low-calling lines, and offspring of low-calling lines demonstrate less anxiety-like behavior compared to the high-calling line (Dichter et al., 1996; Brunelli et al., 1997). In the present study, pups within each litter were characterized as either high or low calling relative to their litter median, and high- and low-calling pups were evenly distributed among treatment conditions.

Pups were weaned from the dam at P22 and placed into same-sex sibling groups (2–4 mice) in standard cages (28 cm × 17 cm × 12 cm) with corn-cob bedding. Cages were not individually ventilated, but rather had standard wire lid covers with a filter top over the lid. Identical weekly husbandry procedures were used for all groups. Mice remained in these groups until P50, at which point they were single-housed in standard cages, then returned to cages with their original littermates on P60. On P110, mice were again single-housed for behavior testing (P120–135) until sacrificed (P140; **Figure 1C**). All mouse cages had a red polypropylene tube, which acted as environmental enrichment and a familiar transport vehicle for experimental manipulations (Roy et al., 2001). Throughout the study, colony rooms were maintained at 21 ± 1°C, at 30%–70% humidity, and on a reverse 12:12 light:dark schedule (lights on 18:00 h, lights off 06:00 h). All animals had *ad libitum* access to food and water throughout the study.

Induction of Adolescent Allergic Asthma Symptoms

Experimental procedures for induction of allergic asthma symptoms were conducted as previously detailed and are briefly described below (Caulfield et al., 2017).

A

Experimental Group	Airway Inflammation Treatment (P7-56)	Labored Breathing Treatment (P22-57)
Control (CON)	Intranasal Saline Control	Inhaled Saline Control
Airway Inflammation (AI)	Intranasal House Dust Mite (HDM)	Inhaled Saline Control
Labored Breathing (LB)	Intranasal Saline Control	Inhaled Aerosolized Methacholine (MCH)
Airway Inflammation + Labored Breathing (AI+LB)	Intranasal House Dust Mite (HDM)	Inhaled Aerosolized Methacholine (MCH)

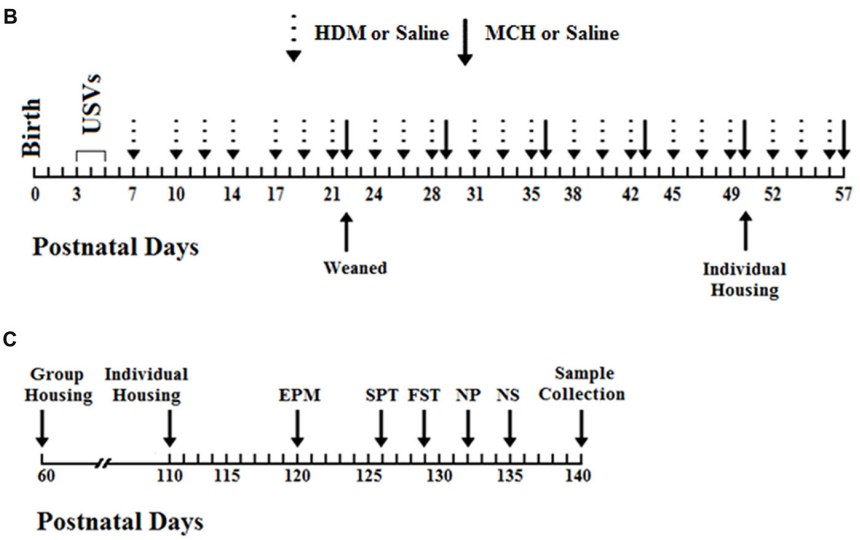

FIGURE 1 | Study Timeline. **(A)** There were four experimental groups in the study, and each group experienced a component of airway inflammation and labored breathing. The first group (Control, CON) served as the control group and received the saline control treatment for both experimental conditions. The second group (Airway Inflammation, AI) was exposed to chronic house dust mite (HDM) to induce inflammation and to the saline control for the labored breathing condition. The third group (Labored Breathing, LB) received saline control for the airway inflammation treatment and methacholine (MCH) to induce labored breathing events. The final group (Airway Inflammation + Labored Breathing, AI+LB) was exposed to both experimental conditions (HDM and MCH). **(B)** Birth was designated as postnatal day (P) zero, and ultrasonic vocalizations (USVs) were conducted from P3–5. AI exposures occurred three times per week from P7–56, and LB treatments occurred once per week from P22–57. **(C)** Mice underwent numerous behavior tests including the elevated plus maze (EPM) on P120, Sucrose Preference Test (SPT) on P126, Forced Swim Test (FST) on P129, Novel Object Task (NP) on P132 and Novel Social (NS) Partner Task on P135. Animals were sacrificed at P140 and samples were collected.

Airway Inflammation (AI)

AI was induced by regularly exposing young mice intranasally to an extract of the most common aeroallergen for humans—house dust mite (*Dermatophagoides pteronyssinus*, HDM; Greer Labs, NC, USA). The AI and AI+LB groups were exposed intranasally to a solution of HDM three times per week, and the CON and LB groups received saline on the same schedule using the same technique (**Figure 1B**). From P7–15, mice received 10 μg (10 μL of 1 mg/ml protein weight solution in saline) of HDM at each exposure, and from P16–56, doses increased to 15 μg HDM (15 μL) and were administered under brief isoflurane anesthesia. This method leads to significant lung inflammation within 2 weeks of first dosage, and elevated inflammation persists throughout the exposure period and at least 3 weeks after

cessation of the HDM exposures (Saglani et al., 2009; Caulfield et al., 2017).

Labored Breathing (LB)

LB was induced by exposure to inhaled methacholine (MCH; Sigma, St. Louis, MO, USA), a muscarinic receptor agonist. From P22–57 exposures occurred once per week (**Figure 1B**). Mice were placed in a whole-body plethysmograph holding chamber (7.5 cm diameter × 7 cm height; Data Sciences International, New Brighton, MN, USA) and allowed to acclimate for 3 min followed by baseline breathing recorded for 3 min. After acclimation and baseline, LB mice were exposed to five increasing doses of aerosolized MCH (0, 6.25, 12.5, 25 and 50 ng/ml in 100 μl saline). AI+LB mice received a half-dose of MCH (0, 3.13, 6.25, 12.5, 25 ng/ml in 100 μl saline) to arrive at LB estimates comparable to LB mice. In prior work, we titrated the best MCH doses for the LB and AI+LB groups to arrive at similar level of LB in both groups. CON and AI mice experienced the exact same procedures but received saline instead of MCH. To verify and estimate extent of bronchoconstriction, enhanced pause (Penh) was recorded (Hamelmann et al., 1997) using FinePointe software. Behavior in the plethysmograph was recorded throughout each session (active, sit still, hunch, LB, drool, gape). If three Penh values were above 15 or if a mouse was visibly distressed, the MCH administration procedure was terminated early. We have previously demonstrated that this procedure leads to significant LB events in both allergen-exposed and -unexposed BALB/cJ mice (Caulfield et al., 2017).

Behavior Testing

Anxiety-Related Behavior, Elevated Plus Maze (EPM)

On P120, elevated plus maze (EPM) was conducted to measure anxiety behavior. This test has been pharmacologically validated, and it is a classic test for observing anxiety-related behavior in mice (Pellow et al., 1985; Lister, 1987; Hogg, 1996; File, 2001; Carobrez and Bertoglio, 2005). The maze consists of two open (30 × 5 cm) and two closed (30 × 14.5 × 5) flat perpendicular arms elevated 42 cm above the ground. Test orders were pseudo-randomized to balance conditions and litter mates. Mice were brought to the test room ∼1 h before testing, transported to the maze in the familiar red tube, and placed in the maze facing an open arm. Testing was completed under red light illumination (<5 lux), and behavior video-recorded for 5 min. Entry into a maze arm was defined as four limbs crossing the boundary between sections. Videos were scored for: percent time spent on open arms, total number of entries into open arms, and total number of entries into open and closed arms. Percent time on and entries into the open arms were used as inverse metrics of anxiety-like behavior, and total arm entries were used to measure overall locomotion.

Hedonic Behavior, Sucrose Preference Test (SPT)

On P126, free-choice consumption of sucrose was recorded in the Sucrose Preference Test (SPT) to examine mouse hedonic behavior (Strekalova et al., 2004). SPT is a reliable measure of depression-related hedonic behavior (Porsolt et al., 1977). Mice had 24-h free access to a bottle with tap water and another bottle with a 3% sucrose solution. BALB/cJ mice show a preference for sucrose solutions with a concentration of sugar that is higher than other inbred mouse strains (Lewis et al., 2005). After 12 h, bottle positions were switched to avoid side preference biases. Prior to and following the 24-h period, each bottle was weighed to calculate consumption of sucrose solution relative to water. Decreased relative sucrose consumption was used as an index of anhedonic behavior (McCormick and Green, 2013).

Depression-Related Behavior, Forced Swim Test (FST)

Forced Swim Test (FST) was conducted on P129 to measure depression-related behavior. Mice were individually tested by placing them into a large beaker of water (25–27°C) for 6 min. Latency to become immobile, number of times immobile, and total time immobile were quantified from video coding. Immobility was defined as lack of movement in at least three limbs. FST is a classic test for depressive behaviors, and higher levels of immobility are indicators of this (McCormick and Green, 2013).

Novelty Exploration, Novel Physical and Novel Social Arenas

Exploratory behavior was measured on two separate arenas, one containing novel mouse-sized objects and another containing a novel social (NS) partner as previously described (Cavigelli et al., 2007). Briefly, both arenas were 120 cm × 120 cm with opaque walls and a Plexiglas cover. The floor was covered with semi-soiled bedding. For the Novel Physical test, small objects were placed in three of the four corners. For the NS test, a same-age, same-sex mouse was placed in a wire container in one corner, and a similar empty container was placed in the opposite corner. For both tests, mice were run individually by carrying them in a red enrichment tube from their home cage to the open corner of the arena. Behavior was video-recorded using a camera positioned well above the arena, and all testing was conducted in low, red light (<10 lux). Latency to approach a novel object or the NS partner were recorded in each arena; these behaviors are associated with stress regulation (Cavigelli et al., 2007).

Physiological Outcomes

Lung Inflammation, Mucus and Collagen

Left and right posterior lung sections were collected, preserved in formalin, embedded in paraffin and then sliced. Consecutive slices were stained with periodic acid-Schiff, hematoxylin and eosin (H&E), or Masson's trichrome to quantify mucus, inflammation and collagen respectively. Mucus levels were quantified on a scale of 0–6 as previously described, with increasing numbers indicating increasing mucus (Caulfield et al., 2017), and an average mucus score was calculated per mouse based on measures from six slices. The number of discrete inflammation areas (clusters of inflammatory cells) and the length of each area were measured perpendicular to airway/vessel membranes (20 μm diameter or larger) as previously described (Caulfield et al., 2017). Total number of these areas and mean length

were calculated from the six largest areas (three from each lung/mouse, or as many areas as possible). Average collagen thickness was quantified for each mouse based on five thickness measures from each of 3–5 airways on each of two lung slices per mouse as previously detailed (Caulfield et al., 2017).

Adult Lung Cytokine Gene Expression

Lungs were collected at sacrifice and stored in RNAlater (Ambion, Carlsbad, CA, USA) for 24-h before freezing at −80°C. Tissue RNA extraction was conducted using TRIzol reagent (Invitrogen; Carlsbad, CA, USA) and Qiagen RNeasy columns (Qiagen, Germantown, MD, USA). RNA quantity and quality were determined with a NanoDrop™ spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and Agilent 2100 BioAnalyzer™ (Agilent Technologies, Santa Clara, CA, USA), respectively. Complementary DNA (cDNA) was reverse transcribed from RNA with High-Capacity cDNA Reverse Transcription kits (Applied Biosystems, Wilmington, DE, USA). Quantitative real time PCR (qRT-PCR) was conducted to measure relative abundance of the following genes in cDNA: *IL-4* (Mm00445259 m1), *IL-5* (Mm00439646 m1). We also conducted PCR to measure *IL-13* expression (Mm00434204 m1), but because of poor amplification, we do not report these results here. Reactions were prepared in 96-well plates in triplicate with validated TaqMan probes on a StepOnePlus RT PCR System (Applied Biosystems). The following cycle settings were implemented: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s and 60°C test for 60 s. Beta actin (*Actb*) was used as the reference gene. Gene expression scores were standardized to the median control mouse, and relative gene abundance in each sample was determined with the $2^{-\Delta\Delta CT}$ method as has been done previously (Caulfield et al., 2017).

Adult Brain Serotonin- and HPA-Related Gene Expression

Brains were freshly dissected at sacrifice, and the following brain regions were collected: brainstem, hippocampus and prefrontal cortex (PFC). All sections were collected, processed and analyzed as described above for lung cytokine gene expression and as described previously (Caulfield et al., 2017). The following TaqMan Gene Expression Assay primers and probes were used for PCR with brain tissue cDNA: *SERT*, serotonin receptor 1a (*5Htr1a*), corticotropin releasing hormone receptor 1 (*Crhr1*) and glucocorticoid receptor (*GR*; Life Technologies, Mm00439391 m1, Mm00434106 s1, Mm00432670 m1 and Mm00433832 m1, respectively). The serotonin system is highly implicated in anxiety and depression-related disorders (Holmes et al., 2003), and this system is known to be affected in models of allergy (Nau et al., 2015; Shajib and Khan, 2015). Corticotropin releasing hormone and GRs are important aspects of the stress response and anxiety-related behavior, and their function can also become altered in response to developmental stress (Contarino et al., 1999; Spear, 2000; McCormick and Green, 2013).

Serum Corticosterone

To measure basal glucocorticoid levels, trunk blood was collected immediately after sacrifice. Mean time required to sacrifice and collect a blood sample after removal from the home cage was 4.3 min (SEM: 0.11). Samples were centrifuged at 15,000 rpm for 15 min at 4°C, and serum collected and stored at −80°C until analysis. Samples were analyzed in duplicate with a commercial [125I] radioimmunoassay kit (MP Biomedicals, Solon OH, USA). Intra-and inter-assay coefficients of variation for a low and high control were 4.72 and 6.92 (for low control) and 5.51 and 6.88 (for high control). Time required for sample collection was not related to serum corticosterone concentration ($r = -0.125$, $p = 0.182$).

Statistical Analyses

To compare behavioral and physiological outcome variables across conditions, ANCOVAs were conducted with AI (intranasal saline vs. HDM exposure), LB (inhaled aerosolized saline vs. MCH exposure), Sex (male vs. female) and USV category (high vs. low) as factors. We used the cohort mean for each outcome variable as a covariate to control for variation between cohorts. Alpha was designated as 0.05. For all statistical tests, variable distribution was examined to verify normal distribution. The following variables were log transformed to achieve a normal distribution for analyses: lung *IL-4* and *IL-5* gene expression, brainstem *SERT* gene expression, serum corticosterone, percent sucrose consumed in the SPT, percent time spent on the open arms of the EPM, latency to immobility in the FST, percent time immobile in the FST, latency to approach an object in the Novel Physical task, and latency to approach a social partner in the NS task. Outliers were defined as ±2.5 SD and removed prior to statistical analyses. Figures detail the untransformed estimated marginal means for clarity. When there were no main or interaction effects of Sex or USV category, we presented means in the figures collapsed across these factors. Repeated measures ANOVAs were used to determine if LB and/or Penh values changed during repeat administrations from P22–57. Correlation analyses were conducted to determine if there were any linear relationships between gene expression (lung or brain), lung function measures and behavioral outcomes.

RESULTS

Adult Physiology (P140)

Lung Cytokine Gene Expression

Three months after the final adolescent HDM and MCH exposures, there was a significant main effect of Sex on *IL-4* and *IL-5* expression—females had elevated levels compared to males (*IL-4*: $F_{(1,69)} = 11.13$, $p < 0.001$, **Figure 2A**; *IL-5*: $F_{(1,68)} = 158.23$, $p < 0.001$, **Figure 2B**). There were no other significant main effects or interactions present for *IL-4* (*F*s < 3.62, *p*s > 0.061). There was a main effect of AI on *IL-5* expression—animals treated with HDM had higher *IL-5* expression than those not treated with HDM ($F_{(1,69)} = 90.24$, $p < 0.001$, **Figure 2B**). There was also a main effect of LB, where MCH-treated mice had

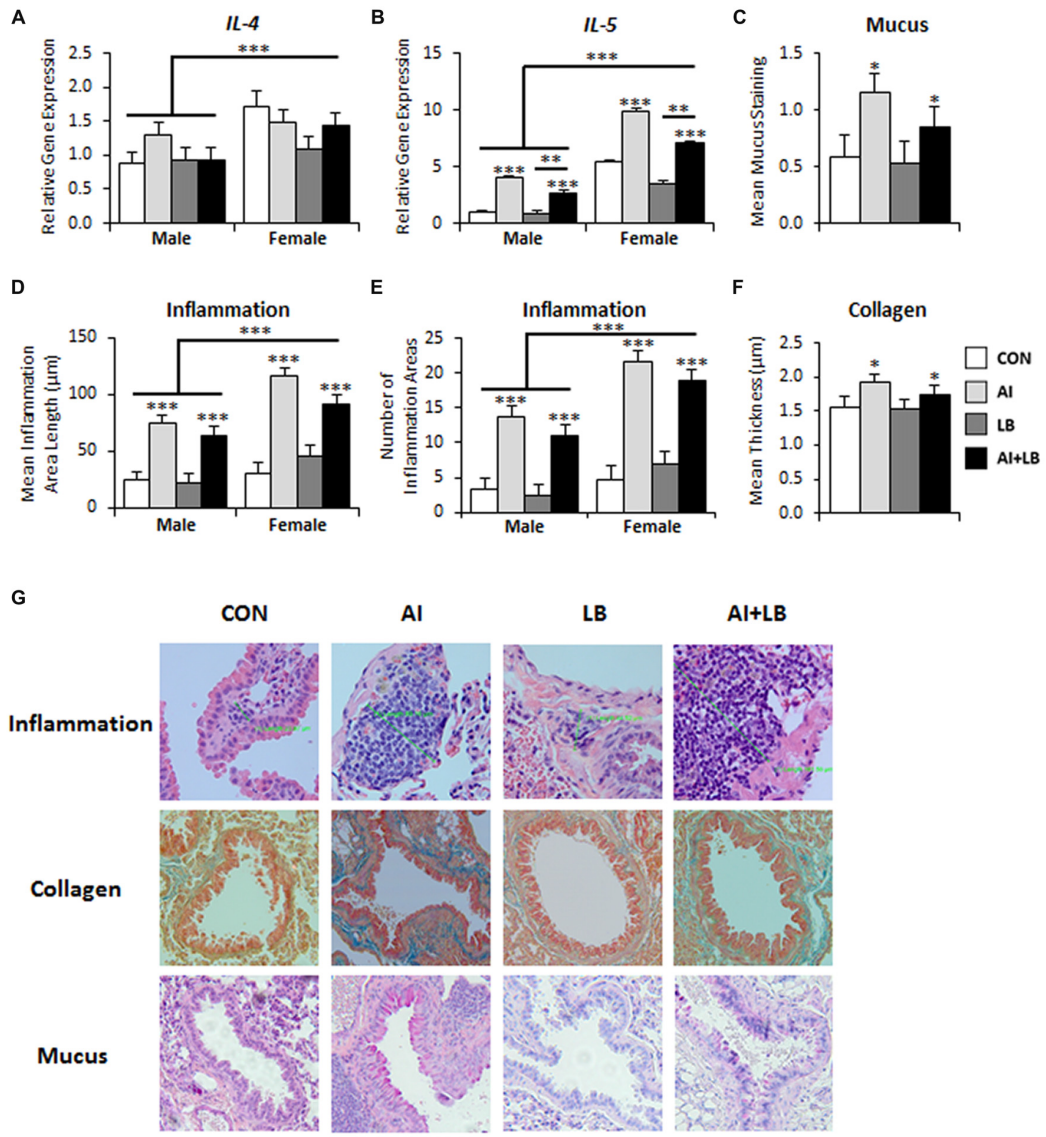

FIGURE 2 | Long-term lung effects of peri-adolescent allergen exposure. **(A)** Interleukin (*IL*)-*4* gene expression was significantly elevated in females compared to males. **(B)** *IL-5* gene expression demonstrated main effects of sex, AI and LB. **(C)** Mucus levels, quantified from PAS-stained lung sections, were elevated in animals that received AI. **(D,E)** Lung inflammation, quantified from hematoxylin and eosin (H&E)-stained sections, indicated that the average inflammation area length **(D)** and number of areas **(E)** were significantly elevated in females compared to males and in AI animals compared to non-AI animals. **(F)** Collagen levels, quantified from Mason trichrome-stained sections, were elevated in animals that received AI. **(G)** Representative images of lung sections stained for inflammation (H&E), mucus (PAS) and collagen (Mason trichrome). Magnification is 20×. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

less *IL-5* expression compared to mice that were not exposed to MCH ($F_{(1,68)} = 9.30$, $p < 0.01$, **Figure 2B**). Finally, there was a significant main effect of USV, where high-calling mice had lower *IL-5* expression than low-calling mice ($F_{(1,68)} = 4.41$, $p < 0.05$; data not shown in figure). No significant interactions were noted for *IL-5* ($Fs < 2.02$, $ps > 0.160$). We had poor amplification for *IL-13* and thus do not report results here.

Lung Mucus

There was a significant effect of AI on mucus levels; mice exposed to chronic peri-adolescent HDM treatments had higher levels of mucus in the lungs 3 months after final allergen treatments ($F_{(1,78)} = 6.07$, $p < 0.05$, **Figure 2C,G**). No other main effects or interactions were significant ($Fs < 3.63$, $ps > 0.060$).

Lung Inflammation

Three months following completion of allergen exposure, mice treated with HDM still had significant symptoms of AI—i.e., greater average length and number of discrete areas of inflammation—compared to non-HDM treated mice (AI main effect on inflammation area length—$F_{(1,78)} = 82.76$, $p < 0.001$; and inflammation area number—$F_{(1,78)} = 100.70$, $p < 0.001$,

Figures 2D,E,G). There was also a main effect of Sex and an interaction of Sex and AI for measures of AI (Sex effect: average length of inflammation area—$F_{(1,78)} = 16.03$, $p < 0.001$, average number of inflammation areas—$F_{(1,78)} = 20.93$, $p < 0.001$, **Figures 2D,E**; Sex × AI interaction: average number of inflammation areas—$F_{(1,78)} = 4.43$, $p < 0.05$). Females had greater inflammation than males, and female-specific increased inflammation was particularly pronounced in the HDM-treated mice. There were no other significant main or interaction effects for average inflammation area length or count (Fs < 3.86, ps > 0.053; Fs < 2.04, ps > 0.157).

Lung Collagen

Mice that were exposed to developmental HDM had significantly more collagen compared to mice that were not exposed to HDM ($F_{(1,71)} = 4.12$, $p < 0.05$; **Figures 2F,G**). There were no other significant main or interaction effects for average collagen thickness (Fs < 3.68, ps > 0.059).

Peri-adolescent Bronchoconstriction (P22–57)

Compared to saline administration, MCH administration caused significantly increased LB counts and Penh values throughout development (LB: $F_{(1,20)} = 170.56$, $p < 0.001$, **Figure 3A**; Penh: $F_{(1,20)} = 130.28$, $p < 0.001$, **Figure 3B**). LB and Penh values increased with age (LB: $F_{(5,350)} = 3.66$, $p < 0.01$; Penh: $F_{(1,100)} = 3.15$, $p < 0.05$), and mice treated with both HDM and MCH (AI+LB) had greater increases in LB and Penh values than mice treated with MCH alone (LB; AI × LB interaction: LB—$F_{(1,20)} = 4.47$, $p < 0.05$; Penh—$F_{(1,20)} = 15.32$, $p < 0.001$; Group means across all ages for LB: CON 0.00 ± 0.19, AI 0.08 ± 0.16, LB 1.12 ± 0.18, AI+LB 1.63 ± 0.18; Penh: CON 0.48 ± 0.32, AI 0.63 ± 0.32, LB 1.34 ± 0.30, AI+LB 2.37 ± 0.30).

Adult Behavior

Elevated Plus Maze (P120)

There was a significant interaction of LB and USV on percent time and number of entries in the open arms of the EPM. For high-calling mice, MCH-exposure led to more time spent and more entries in the open arms. For low-calling mice, MCH-exposure led to less time spent and fewer entries into the open arms relative to unexposed mice (LB × USV interaction: percent time in open arms—$F_{(1,80)} = 8.05$, $p < 0.01$; and number of entries to open arms—$F_{(1,80)} = 4.97$, $p < 0.05$, **Figures 4A,B**). Additionally, there was a three-way interaction between AI, LB and USV for time spent on the open arms, where the MCH effect described above was dampened in mice exposed to both HDM and MCH ($F_{(1,80)} = 5.01$, $p < 0.05$, note final AI+LB bar in **Figure 4A**). No other significant main or interaction effects were observed for time spent or entries on open arms of EPM (Fs < 2.79, ps > 0.099; Fs < 2.81, ps > 0.098). Time spent on the open arms and number of entries on open arms were significantly correlated ($r = 0.782$, $p < 0.001$). No significant main effects or interactions were observed in total arm entries in the EPM (Fs < 2.98, ps > 0.088; **Figure 4C**).

Latency to Approach Novelty (P132, P135)

In the novel object test (P132), there was a significant main effect of Sex—males took longer to approach a novel object compared to females ($F_{(1,66)} = 4.97$, $p < 0.05$; **Figure 4D**). There was also a significant three-way interaction between AI, LB and USV ($F_{(1,81)} = 4.15$, $p < 0.05$); HDM and MCH independently decreased adult approach latencies for high-calling mice and increased latency time in low-calling mice. Both effects were negated by exposure to both HDM and MCH. There were no other significant effects or interactions between groups to approach a novel object (Fs < 1.32, ps > 0.255). There were no significant main effects or interactions in latency to approach a novel partner (P135; Fs < 2.29, ps > 0.135).

Forced Swim Test (P129)

Adult mice that were exposed to HDM during development became immobile faster in the FST compared to mice that were not exposed ($F_{(1,80)} = 5.68$, $p < 0.05$, **Figure 4E**). There were no other main or interaction effects for latency to immobility (Fs < 2.51, ps > 0.117). There were no significant main effects or interactions for percent time spent immobile in the FST (Fs < 2.64, ps > 0.108). There was a significant three-way interaction between Sex, AI and USV for number of times immobile ($F_{(1,81)} = 5.37$, $p < 0.05$). HDM-exposure caused high-calling males and low-calling females to increase the number of immobility bouts in the FST compared to similar calling males and females that were not exposed to HDM. No other main effects or interactions were found for number of times immobile (Fs < 2.77, ps > 0.100).

Sucrose Preference Test (P126)

Analysis of percent sucrose consumed in the SPT revealed a significant interaction between Sex and USV; high-calling female mice consumed less sucrose than low-calling females, whereas high-calling males consumed more sucrose solution than low-calling males ($F_{(1,80)} = 4.09$, $p < 0.05$). No other main effects or interactions were observed for percent sucrose consumed (Fs < 2.54, ps > 0.115; **Figure 4F**).

Basal Corticoid Rhythm (P140)

Circulating basal corticosterone levels were measured at time of sacrifice (P140), 3 months following HDM/MCH exposure. There were no main or interaction effects on adult circulating corticosterone concentrations (Fs < 2.44, ps > 0.123; experimental group means: CON 47.53 ± 12.48, AI 48.88 ± 11.14, LB 40.01 ± 12.32, AI+LB 55.82 ± 12.08).

Adult Brain Gene Expression (P140)

Three months after the end of peri-adolescent asthma treatments, females that had been exposed to HDM during development had greater *SERT* expression in the brainstem, whereas HDM-exposed males had diminished *SERT* expression compared to non-HDM exposed mice (Sex × AI interaction—$F_{(1,81)} = 6.53$, $p < 0.05$, **Figure 5A**). There was also a three-way interaction of AI, LB and USV, such that high-calling pups exposed to either HDM or MCH

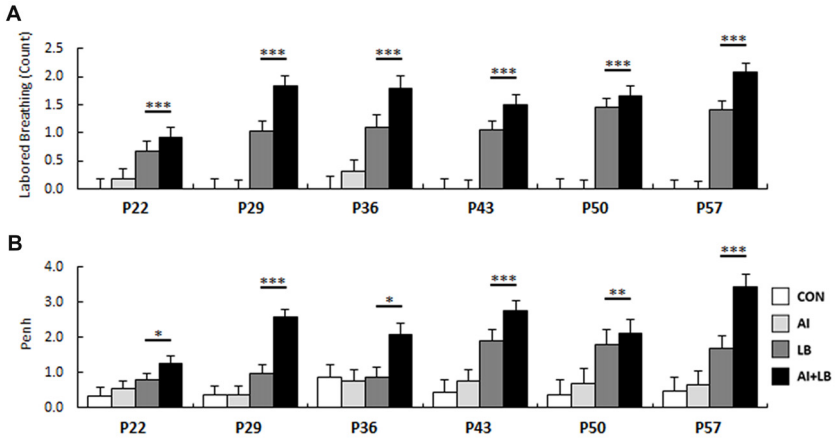

FIGURE 3 | Developmental bronchoconstriction. Mean **(A)** LB response and **(B)** Penh value for mice exposed to MCH was significantly increased compared to mice that were exposed to saline. This was evident at each age of administration. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

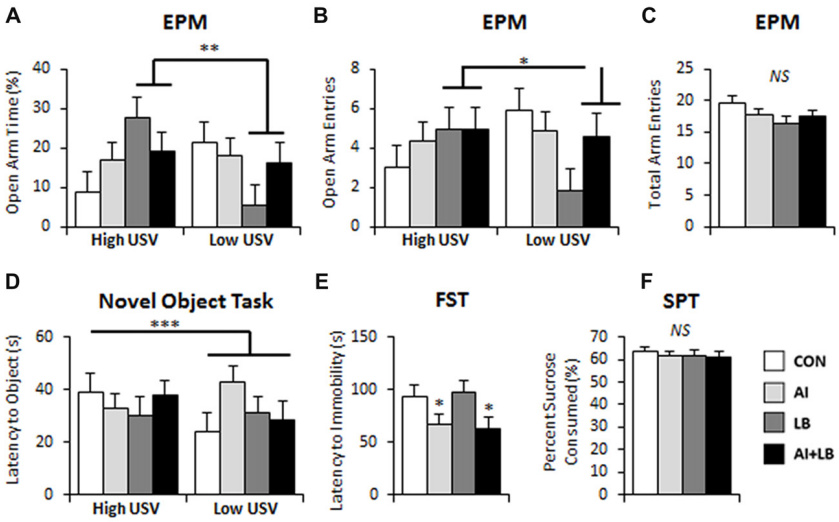

FIGURE 4 | Long-term behavioral effects of peri-adolescent allergic asthma symptoms. **(A,B)** High-calling mice (based on USV) that also experienced developmental LB showed less anxiety-like behavior on the EPM compared to low-calling mice in terms of time spent on the open arms of the maze **(A)** and number of entries made into the open arms of the maze **(B)**. **(C)** There were no significant differences evident in number of total arm entries made in the EPM. **(D)** In the novel object task, low-calling mice demonstrated a faster latency to approach a novel object compared to mice that were categorized as high-callers. **(E)** AI animals demonstrated faster latency to immobility in the FST compared to those that did not experience AI. **(F)** No significant main effects were observed in the sucrose preference test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, *NS*, not significant.

showed increased *SERT* expression, whereas low-calling pups had increased *SERT* expression only if they had received both HDM and MCH ($F_{(1,81)} = 4.70$, $p < 0.05$). There were no other main or interaction effects on *SERT* expression ($Fs < 2.72$, $p > 0.103$). Females had higher *5HTr1a* expression in PFC than males ($F_{(1,69)} = 5.31$, $p < 0.05$, **Figure 5B**). No other main effects or interactions were significant for PFC *5HTr1a* expression ($Fs < 2.10$, $p > 0.151$). For hippocampal *5HTr1*a expression, there were no main or interaction effects ($Fs < 1.38$, $p > 0.244$). There was a significant three-way interaction of Sex, AI and USV on *Crhr1* expression in PFC; relative to control mice, HDM-treated low-calling females had greater expression than HDM-treated low-calling males ($F_{(1,67)} = 5.02$, $p < 0.05$, **Figure 5C**; three-way interaction not shown on figure). No other interactions or main effects were significant ($Fs < 2.11$, $p > 0.151$). For high-calling mice, developmental HDM exposure resulted in decreased hippocampal *GR* expression in adulthood, whereas the reverse was true for low-calling mice (AI × USV interaction—$F_{(1,69)} = 5.52$, $p < 0.05$, **Figure 5D**). No other main effects or interactions were significant ($Fs < 3.90$, $p > 0.052$).

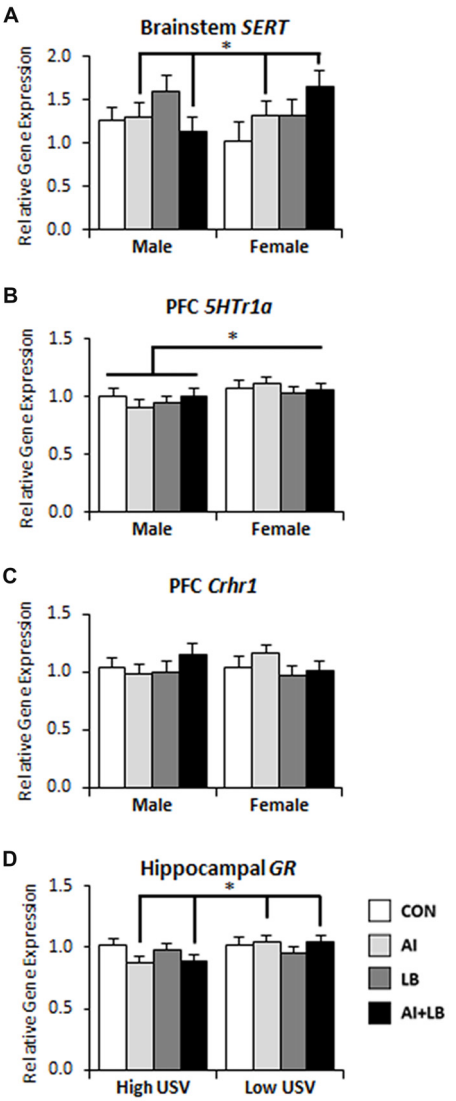

FIGURE 5 | Long-term brain gene expression effects of peri-adolescent allergic asthma symptoms. **(A)** Females that experienced developmental AI had higher serotonin transporter (*SERT*) gene expression in brainstem compared to males that experienced AI in development. **(B)** Females had higher levels of serotonin receptor 1a (*5Htr1a*) gene expression in prefrontal cortex (PFC) compared to males 3 months after asthma symptom exposures had been completed. **(C)** PFC corticotropin releasing hormone receptor 1 (*Crhr1*) gene expression in male and female mice across asthma condition; HDM-treated low-calling females had more expression than HDM-treated low-calling males (this three-way interaction is not indicated in the figure). **(D)** High-calling mice that experienced AI had decreased glucocorticoid receptor (*GR*) gene expression in hippocampus compared to low-calling mice that experienced AI. *$p < 0.05$.

Correlations

Lung Measures

Many of the measures of lung inflammation and function were significantly correlated with one another (correlation statistics in **Table 1**). *IL-5* expression was significantly and positively correlated with average inflammation area length ($r = 0.694$, $p < 0.001$), inflammation area count ($r = 0.661$, $p < 0.001$) and mucus ($r = 0.275$, $p = 0.011$). Average inflammation area length was strongly and positively correlated with inflammation area count ($r = 0.913$, $p < 0.001$), and mucus was positively correlated with inflammation area length and count ($r = 0.395$, $p < 0.001$; $r = 0.359$, $p < 0.001$). Collagen was positively correlated with inflammation patch count ($r = 0.211$, $p = 0.050$), but it was not correlated with average patch length ($r = 0.188$, $p = 0.140$), mucus ($r = 0.188$, $p = 0.082$), or *IL-5* gene expression in lungs ($r = 0.112$, $p = 0.333$).

Lungs and Anxiety- and Depression-Related Behavior

Some lung measures were significantly correlated with behavior (**Table 1**). Percent time spent on the open arms of the EPM was positively correlated with average inflammation area length ($r = 0.213$, $p < 0.05$) and mucus ($r = 0.324$, $p < 0.001$), and marginally related to inflammation area count ($r = 0.187$, $p = 0.071$). Open arm entries on the EPM were positively correlated with average inflammation area length ($r = 0.212$, $p < 0.05$), inflammation area count ($r = 0.220$, $p < 0.05$), and mucus ($r = 0.232$, $p < 0.05$). However, there were no significant correlations between depression-related behavior (latency to immobility on the FST) and the following lung measures: average inflammation area length ($r = -0.046$, $p = 0.661$), number of inflammation areas ($r = -0.086$, $p = 0.408$), mucus ($r = -0.001$, $p = 0.993$) and lung *IL-5* expression ($r = 0.155$, $p = 0.157$).

Brain Gene Expression and Anxiety-Related Behavior

A few correlations were found between brain gene expression and anxiety-related behavior (**Table 1**). *5HTr1a* gene expression in PFC was significantly positively correlated with percent time spent on the open arms of the EPM ($r = 0.324$, $p < 0.01$) and number of open arm entries in the EPM ($r = 0.234$, $p < 0.05$). It was also negatively correlated with mean USV calling rate ($r = -0.294$, $p < 0.01$). On the other hand, *5HTr1a* expression in the hippocampus was not correlated with these same behavioral measures ($r = 0.108$, $p = 0.326$; $r = 0.095$, $p = 0.386$; $r = 0.163$, $p = 0.100$).

DISCUSSION

Long-Term Behavior and Brain Changes Following Peri-adolescent Asthma

Results of the current study indicate that chronic inhaled allergen exposure during development led to long-term changes in lung function. Exposure to HDM extract three times per week from neonatal age to late adolescence led to increased AI, mucus, collagen and *IL-5* gene expression 3 months after final allergen exposure, particularly in females. In addition, developmental allergen exposure (and associated lung alterations) altered gene expression for brainstem *SERT* and PFC *Crhr1*, with these effects being sex- and USV-specific. Females that had been exposed to allergen during development showed increased brainstem *SERT* expression, and

TABLE 1 | Statistical correlations among lung physiology, gene expression and behavior.

Factor 1	Factor 2	Sample size	Pearson correlation (*r*)	Significance (*p*)
Lung *IL-5* Expression	Inflammation Area Length	85	0.694	**<0.001**
Lung *IL-5* Expression	Inflammation Area Count	85	0.661	**<0.001**
Lung *IL-5* Expression	Mucus	85	0.275	**0.011**
Inflammation Area Count	Inflammation Area Length	95	0.913	**<0.001**
Mucus	Inflammation Area Length	95	0.395	**<0.001**
Mucus	Inflammation Area Count	95	0.359	**<0.001**
Collagen	Inflammation Area Count	87	0.211	**0.050**
Collagen	Inflammation Area Length	87	0.160	0.140
Collagen	Mucus	87	0.188	0.082
Collagen	*IL*-5 Gene Expression	77	0.112	0.333
EPM % Time on Open Arms	Inflammation Area Length	94	0.213	**0.039**
EPM % Time on Open Arms	Inflammation Area Length	94	0.187	0.071
EPM % Time on Open Arms	Mucus	94	0.324	**0.001**
EPM Open Arm Entries	Inflammation Area Length	94	0.212	**0.040**
EPM Open Arm Entries	Inflammation Area Length	94	0.220	**0.033**
EPM Open Arm Entries	Mucus	94	0.232	**0.025**
FST Latency to Immobility	Inflammation Area Length	95	−0.046	0.661
FST Latency to Immobility	Inflammation Area Count	95	−0.086	0.408
FST Latency to Immobility	Mucus	95	−0.001	0.993
FST Latency to Immobility	*IL*-5 Gene Expression	85	0.155	0.157
PFC *5HTr1a* Expression	EPM % Time on Open Arms	85	0.324	**0.002**
PFC *5HTr1a* Expression	EPM Open Arm Entries	85	0.234	**0.031**
PFC *5HTr1a* Expression	Mean USV Calling Rate	102	−0.294	**0.003**
Hippocampus *5HTr1a* Expression	EPM % Time on Open Arms	85	0.108	0.326
Hippocampus *5HTr1a* Expression	EPM Open Arm Entries	85	0.095	0.386
Hippocampus *5HTr1a* Expression	Mean USV Calling Rate	103	0.163	0.100

Bold values indicate significant correlations.

low-calling females showed increased PFC *Crhr1* expression, compared to non-exposed females. Allergen-exposed males, on the other hand, showed decreased brainstem *SERT* expression and more modest increases in PFC *Crhr1* expression compared to unexposed males. These results, 3 months after final asthma symptom induction, contrast with previously observed results on short-term responses to developmental allergen exposure. Specifically, in a prior study, we found that 3 weeks after symptom induction was completed, there were no significant effects of HDM exposure on similar adult anxiety- or depression-related behaviors or similar brain gene expression related to emotion and stress regulation. Rather, weekly exposure to MCH to induce LB led to adult anxiety-related behavior and brain gene expression in the short-term (Caulfield et al., 2017). This disparity suggests that allergen exposure during development, which causes immediate and significant AI, mucus and collagen buildup, does not have immediate effects on behavior and brain function, but rather, that long-term allergic asthma symptoms that persist during development and adulthood may eventually affect later behavior and brain function.

While developmental allergen exposure caused several changes in the above behavior and brain gene expression profiles, the experimentally-induced, repeat, acute bronchoconstriction events during development had fewer long-term effects on behavior and brain gene expression. Exposure to inhaled MCH once per week, which caused significant increases in LB and Penh values at the time of exposure, only led to one long-lasting effect on behavior and no long-lasting effects on gene expression in the current study. The long-lasting behavioral effect of peri-adolescent LB was increased anxiety-related behavior on the EPM for mice that were low USV-callers (i.e., low fear) as neonates. In a prior study, we found that developmental LB led to significant short-term changes in anxiety-related behavior and brain gene expression; specifically, developmental MCH exposure caused decreased open arm time on the EPM, decreased brainstem *SERT* expression, and increased hippocampal *5Htr1a* and *Crhr1* expression 3 weeks after final MCH exposure (Caulfield et al., 2017). This difference in results between the current and prior study suggest that repeat exposure to acute LB events during development may lead to significant short-term changes in anxiety-like behavior and brain gene expression, and that these effects subside over time. Long-term anxiety-like behavior may only persist in individuals that initially show relatively low levels of fear. Overall, the results of the current study suggest that the strongest long-term impacts of developmental asthma on behavior and brain function may depend on persistent effects to lung function that result from chronic allergen exposure during development, as opposed to long-term behavior/brain changes that result from a discrete developmental period of allergic asthma symptoms.

In the current study, we also documented significant effects of sex and neonatal USV rates on adult lung and anxiety-related outcomes. Females showed more signs of lung inflammation and *IL-4* and *IL-5* expression than males—an effect that has been previously documented (Blacquière et al., 2010; Caulfield et al., 2017). Females also displayed greater exploration (i.e., faster latency to approach a novel object) compared to males. Females

also had more *5HTr1a* expression in the PFC compared to males. It has been previously noted that females have higher rates of anxiety- and mood-related disorders compared to males (Roza et al., 2003; Ross et al., 2007; Holder and Blaustein, 2014). Some of these female-specific results of the present study were also evident in adult mice that, as pups, had displayed less fear-related USVs when isolated. Adult mice that displayed low-calling USV rates as pups had increased *IL-5* expression in the lungs. Pup ultrasonic calling rates also modulated some of the effects of developmental allergen and LB on adult behavior. Regardless of sex, mice that experienced weekly MCH exposures spent more time and made more entries onto the open arms of the EPM if they were high callers rather than low callers. Additionally, low-calling mice approached a novel object faster than high-calling mice in the Novel Physical test. USVs are vocal signals produced by pups in various ethologically important circumstances including isolation or separation from the nest, and they are important in mother-pup communication in early life (Bell et al., 1972; Branchi et al., 2001). In rodents, USVs can be predictors of later-life emotion regulation; mice selectively bred based on pup frequency of USVs develop into distinct high and low-calling lines, and offspring of low-calling lines demonstrate less anxiety-like behavior compared to the high-calling line (Dichter et al., 1996; Brunelli et al., 1997). In 7-day old mice, USV production can be modulated by anxiolytic and anxiogenic drugs (Fish et al., 2004; Takahashi et al., 2009). The present results are in contrast to what would be expected—if high rates of USVs are suggestive of a higher predisposition to become anxious, it would be expected that those mice would demonstrate higher levels of inflammation and anxiety-related behavior compared to low-calling mice. However, in the current study, mice that showed low-calling rates demonstrated higher levels of lung *IL-5* gene expression, more anxiety-related behavior, longer latencies to approach novelty and increased hippocampal *GR* gene expression. These results are in the opposite direction of what is expected, and they suggest that low-callers, when faced with a challenge, increase their inflammatory and anxiety-like symptoms. On the other hand, high-calling mice may have more resources to respond to developmental challenges and show fewer long-term adult consequences of adolescent stressors.

Persistent Alteration to Lung Function After Developmental Immune Challenges

Previous research has demonstrated how early life respiratory events alter later life lung function. For example, in mice, neonatal exposure to high concentrations of oxygen causes changes in lung development that persists into adulthood (Yee et al., 2009). Human data also indicate that children who have persistent or severe asthma are more likely to continue experiencing irregular lung function as adults and are at higher risk for developing COPD (Pasterkamp et al., 1997; Tai et al., 2014b). Additionally, children that experience pneumonia in early life (before 3 years of age) have impaired lung function in adolescence and adulthood compared to subjects that never had pneumonia during this time (Chan et al., 2015). In the current study, persistent lung alterations following peri-adolescent allergen exposure may have accounted for increased immobility in the FST, a classic test for depression-related behaviors (McCormick and Green, 2013). HDM-exposed mice still showed significantly elevated lung inflammation, mucus and collagen levels at the time of FST testing, which occurred 3 months after termination of allergen exposure. More rapid and frequent immobility in the FST was likely an effect of the persistent AI and associated decreased oxygen availability for HDM-exposed mice, as opposed to "depression-like" symptoms *per se*.

Other studies have demonstrated lasting airway inflammatory processes in rodent asthma models. For example, LACK peptide (a novel antigen) exposure used to induce asthma symptoms in BALB/cAnN mice, beginning at 6 weeks of age, led to inflammatory symptoms that persisted 5 and 8 weeks after the termination of antigen exposure (Julia et al., 2002). In adult female BALB/cJ mice, intranasal exposure to ovalbumin for 12 weeks led to significant eosinophilic inflammation, goblet cell hyperplasia and collagen deposition that resolved 4 weeks after final allergen exposure, and lymphocyte inflammation and smooth muscle thickening took 8 weeks to resolve (Alrifai et al., 2014). Adult female BALB/cJ mice exposed to ovalbumin periodically over a 55-day protocol demonstrated significant inflammation but no airway hyper-responsiveness 1 month after ovalbumin exposure was terminated (McMillan and Lloyd, 2004). Adult female BALB/c mice exposed to ovalbumin every other day, over an 8-week period, had lasting inflammation 2, 4, 6 and 8 weeks following the termination of allergen exposure (Temelkovski et al., 1998). In the present study, we demonstrated that 8 weeks of peri-adolescent exposure to HDM led to persistent AI 11.5 weeks after the end of allergen exposure, and that this inflammation was more pronounced in females compared to males. These results were evident in histological measures and in cytokine-related gene expression. These persistent effects are notably longer than previously documented persistent effects in adult mice, suggesting that allergic processes that develop during childhood/adolescence may take longer to resolve than allergic processes that begin in adulthood.

In the present study, we demonstrate that lung inflammation, mucus, collagen and allergic cytokine gene expression (*IL-4* and *IL-5*) are increased in adult mice 3 months after the completion of chronic developmental allergen exposures. We have previously documented more enhanced increases in inflammation, mucus and lung gene expression 3 weeks after the completion of asthma symptom exposure, but in this prior study there was no collagen buildup at this early time point (Caulfield et al., 2017). In the current study, airway remodeling, as indicated with collagen buildup, was evident 3 months after completion of asthma symptom exposures. These results suggest that some aspects of lung function (inflammation, mucus, gene expression) persist for a long time after allergen exposure, while aspects related to lung structure (collagen build-up) require a longer time to fully form (Tanaka et al., 2002; Antunes et al., 2010; Salmond et al., 2012).

Limitations and Future Directions

One limitation of the current study involves the dosing of MCH between the LB and the AI+LB treatment groups. In order to create similar LB and Penh values and to be conscious of humane endpoints for the mice in the MCH administration sessions, the dose of MCH (the bronchoconstriction agent) was halved in mice exposed to both HDM and MCH (i.e., the AI+LB group). Based on results from the current study, it appears that in the AI+LB group, lung inflammation, gene expression and behavior were qualitatively different from groups that received only AI or only LB. Although, we found very few statistical interactions of AI and LB, it is important to note that the difference in MCH dosing in the AI+LB group may limit the interpretation of results. Future work should establish a better treatment protocol to induce both AI and LB to understand synergistic effects of these asthma symptoms.

Another potential limitation in the present study involved the 2-min isolation method used to measure neonatal USV rates. While brief isolation causes a stress response in the pup, all pups experienced the same procedure, which was conducted prior to manipulation of asthma symptoms. It is possible that this early-life stress experience, prior to asthma manipulations, may have masked or accentuated the asthma effects reported here. However, this procedure allowed us to control for pre-asthma anxiety-related behavior in individual mice and to determine if early asthma symptoms lead to elevated anxiety-like symptoms in individuals that are otherwise predisposed toward anxiety. Further, we also used multiple behavioral outcome measures in the current study. For an initial exploratory study on potential long-term behavioral effects of allergic asthma, we felt it was important to include multiple behavioral outcomes. However, it is important acknowledge that mouse responses to the latter tests (e.g., forced swim and novelty exploration) may have been affected by earlier testing experiences.

The findings from this study have important implications for research on asthma therapy as it relates to anxiety- and depression-related disorders. This is particularly true for children and adolescents that mature with asthma symptoms and inflammation and need to treat symptoms with chronic pharmaceutical regimes. Specific asthma treatments may differentially influence mechanisms associated with anxiety and/or depression, and these effects should be evaluated in pre-clinical research. For example, many asthma patients are treated with daily inhaled corticosteroids to control asthma symptoms, and these treatments are effective in reducing inflammation (Lee et al., 2008; Alrifai et al., 2014). These drugs also lead to lasting effects on growth, adrenal function, and other processes (Merkus et al., 1993; Hanania et al., 1995; Molimard et al., 2008). However, little is known about how these treatments may affect internalizing disorder susceptibility and associated mechanisms. The current study provides a model in which to test the long-term influence of asthma treatments on both peripheral lung processes and more centralized mechanisms associated with anxiety- and depression-related symptoms. With this initial study completed, future studies can include fewer extraneous control measures in order to minimize potential confounds of early life stress effects on the developing immune system. With fewer early stressors, future studies can avoid experimental noise and provide a stronger signal for interpretation.

CONCLUSION

In summary, the current study is the first to show that persistent lung inflammation coincides with changes in brain gene expression that are associated with emotion and stress regulation, providing potential mechanisms by which developmental asthma may increase risk for internalizing disorders in a rodent model. This study also demonstrates that adolescent allergen-induced lung inflammation, mucus and collagen buildup persist several months after termination of allergen exposure. An important caveat is that these long-term lung, brain and behavior responses to developmental allergic asthma may differ for males and females and may also differ depending on early life temperament/traits. Future work is required to further identify and test potential mechanisms, to determine the influence of asthma treatments, and to identify processes that predispose some individuals with developmental asthma to internalizing disorders.

AUTHOR CONTRIBUTIONS

JC and SC performed statistical analyses and drafted the manuscript. MC, AA, RBourne, NC, TC and LK made editorial contributions to the manuscript. SC, NC, RBourne and MC were involved in data collection. AA, TC, LK, RBonneau and SC contributed ideas to the research funding proposal and research design.

FUNDING

This work was supported by the National Institutes of Health (1R21MH092667-01A1) and the Pennsylvania State Institute for Neuroscience. This work was supported, in part, by NIH Grant T32GM108563.

ACKNOWLEDGMENTS

We would like to acknowledge the intense assistance of many students in the Behavioral Neuroendocrinology Lab: A.G. Agasar, S.J. Allen, A.D. Bao, A.K. Bossert, A.P. Bruscke, D.C. Cardell, J.T. Carp, H.S. Chaudhry, S.L. Cooperstein, T.L. Coppage, E.M. Cover, K. Craig, M. DeNicola, C.G. Firely, J.L. Fox, O.M. Francois, S. Gnanarajah, I.I. Guo, I.M. Kaplan, A. Kech, S.Z. Kidder, H.O. Knisley, S.M. Koo, E.L. Mercier, K.C. Michael, A.C. Motchenbacher, C.M. Ragan, A.J. Rodriguez, J.D. Senville, M.H. Woehling and J.A. Wisniewski. We dedicate this article to RBonneau, esteemed colleague and supportive mentor, who passed away before manuscript completion. This work would not have been possible without him. He is greatly missed.

REFERENCES

Abikoff, H. B., Jensen, P. S., Arnold, L. L. E., Hoza, B., Hechtman, L., Pollack, S., et al. (2002). Observed classroom behavior of children with ADHD: relationship to gender and comorbidity. *J. Abnorm. Child Psychol.* 30, 349–359. doi: 10.1023/A:1015713807297

Ahmad, T., Kumar, M., Mabalirajan, U., Pattnaik, B., Aggarwal, S., Singh, R., et al. (2012). Hypoxia response in asthma: differential modulation on inflammation and epithelial injury. *Am. J. Respir. Cell Mol. Biol.* 47, 1–10. doi: 10.1165/rcmb.2011-0203OC

Akinbami, L. J., Moorman, J. E., Bailey, C., Zahran, H. S., King, M., Johnson, C. A., et al. (2012). Trends in asthm a prevalence, health care use and mortality in the United States, 2001–2010. *NCHS Data Brief* 94, 1–8. doi:10.1186/s12876-017-0681-y

Alrifai, M., Marsh, L. M., Dicke, T., Kiliç, A., Conrad, M. L., Renz, H., et al. (2014). Compartmental and temporal dynamics of chronic inflammation and airway remodelling in a chronic asthma mouse model. *PLoS One* 9:e85839. doi: 10.1371/journal.pone.0085839

Andersen, S. L., and Teicher, M. H. (2000). Sex differences in dopamine receptors and their relevance to ADHD. *Neurosci. Biobehav. Rev.* 24, 137–141. doi: 10.1016/s0149-7634(99)00044-5

Anderson, H. R., Pottier, A. C., and Strachan, D. P. (1992). Asthma from birth to age 23: incidence and relation to prior and concurrent atopic disease. *Thorax* 47, 537–542. doi: 10.1136/thx.47.7.537

Antunes, M. A., Abreu, S. C., Silva, A. L., Parra-Cuentas, E. R., Ab'Saber, A. M., Capelozzi, V. L., et al. (2010). Sex-specific lung remodeling and inflammation changes in experimental allergic asthma. *J. Appl. Physiol.* 109, 855–863. doi: 10.1152/japplphysiol.00333.2010

Barnum, C. J., Pace, T. W. W., Hu, F., Neigh, G. N., and Tansey, M. G. (2012). Psychological stress in adolescent and adult mice increases neuroinflammation and attenuates the response to LPS challenge. *J. Neuroinflammation* 9:9. doi: 10.1186/1742-2094-9-9

Bell, R. W., Nitschke, W., and Zachman, T. A. (1972). Ultra-sounds in three inbred strains of young mice. *Behav. Biol.* 7, 805–814. doi: 10.1016/s0091-6773(72)80172-x

Blacquière, M. J., Hylkema, M. N., Postma, D. S., Geerlings, M., Timens, W., and Melgert, B. N. (2010). Airway inflammation and remodeling in two mouse models of asthma: comparison of males and females. *Int. Arch. Allergy Immunol.* 153, 173–181. doi: 10.1159/000312635

Bourke, C. H., and Neigh, G. N. (2011). Behavioral effects of chronic adolescent stress are sustained and sexually dimorphic. *Horm. Behav.* 60, 112–120. doi: 10.1016/j.yhbeh.2011.03.011

Branchi, I., Santucci, D., and Alleva, E. (2001). Ultrasonic vocalisation emitted by infant rodents: a tool for assessment of neurobehavioural development. *Behav. Brain Res.* 125, 49–56. doi: 10.1016/s0166-4328(01)00277-7

Branchi, I., Santucci, D., Vitale, A., and Alleva, E. (1998). Ultrasonic vocalizations by infant laboratory mice: a preliminary spectrographic characterization under different conditions. *Dev. Psychobiol.* 33, 249–256. doi: 10.1002/(sici)1098-2302(199811)33:3<249::aid-dev5>3.0.co;2-r

Brunelli, S. A., Vinocur, D. D., Soo-Hoo, D., and Hofer, M. A. (1997). Five generations of selective breeding for ultrasonic vocalization (USV) responses in N:NIH strain rats. *Dev. Psychobiol.* 31, 255–265. doi: 10.1002/(sici)1098-2302(199712)31:4<255::aid-dev3>3.0.co;2-q

Buske-Kirschbaum, A., Ebrecht, M., Kern, S., Gierens, A., and Hellhammer, D. H. (2008). Personality characteristics in chronic and non-chronic allergic conditions. *Brain Behav. Immun.* 22, 762–768. doi: 10.1016/j.bbi.2007.12.002

Carobrez, A. P., and Bertoglio, L. J. (2005). Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. *Neurosci. Biobehav. Rev.* 29, 1193–1205. doi: 10.1016/j.neubiorev.2005.04.017

Caruso, M. J., Reiss, D. E., Caulfield, J. I., Thomas, J. L., Baker, A. N., Cavigelli, S. A., et al. (2017). Adolescent chronic variable social stress influences exploratory behavior and nicotine responses in male, but not female, BALB/cJ mice. *Brain Res. Bull.* 138, 37–49. doi: 10.1016/j.brainresbull.2017.08.001

Caulfield, J. I., Caruso, M. J., Michael, K. C., Bourne, R. A., Chirichella, N. R., Klein, L. C., et al. (2017). Peri-adolescent asthma symptoms cause adult anxiety-related behavior and neurobiological processes in mice. *Behav. Brain Res.* 326, 244–255. doi: 10.1016/j.bbr.2017.02.046

Cavigelli, S. A., Stine, M. M., Kovacsics, C., Jefferson, A., Diep, M. N., and Barrett, C. E. (2007). Behavioral inhibition and glucocorticoid dynamics in a rodent model. *Physiol. Behav.* 92, 897–905. doi: 10.1016/j.physbeh.2007.06.016

Chaby, L. E., Cavigelli, S. A., Hirrlinger, A. M., Caruso, M. J., and Braithwaite, V. A. (2015). Chronic unpredictable stress during adolescence causes long-term anxiety. *Behav. Brain Res.* 278, 492–495. doi: 10.1016/j.bbr.2014.09.003

Chan, J. Y. C., Stern, D. A., Guerra, S., Wright, A. L., Morgan, W. J., and Martinez, F. D. (2015). Pneumonia in childhood and impaired lung function in adults: a longitudinal study. *Pediatrics* 135, 607–616. doi: 10.1542/peds.2014-3060

Chida, Y., Hamer, M., and Steptoe, A. (2008). A bidirectional relationship between psychosocial factors and atopic disorders: a systematic review and meta-analysis. *Psychosom. Med.* 70, 102–116. doi: 10.1097/psy.0b013e31815c1b71

Chida, Y., Sudo, N., Sonoda, J., Hiramoto, T., and Kubo, C. (2007). Early-life psychological stress exacerbates adult mouse asthma via the hypothalamus-pituitary-adrenal axis. *Am. J. Respir. Crit. Care Med.* 175, 316–322. doi: 10.1164/rccm.200607-898oc

Contarino, A., Dellu, F., Koob, G. F., Smith, G. W., Lee, K. F., Vale, W., et al. (1999). Reduced anxiety-like and cognitive performance in mice lacking the corticotropin-releasing factor receptor 1. *Brain Res.* 835, 1–9. doi: 10.1016/s0006-8993(98)01158-5

Dahl, R. E. (2004). Adolescent brain development: a period of vulnerabilities and opportunities. *Ann. N Y Acad. Sci.* 1021, 1–22. doi: 10.1196/annals.1308.001

Dichter, G. S., Brunelli, S. A., and Hofer, M. A. (1996). Elevated plus-maze behavior in adult offspring of selectively bred rats. *Physiol. Behav.* 60, 299–304. doi: 10.1016/0031-9384(95)02222-8

Dong, H., Zhang, X., and Qian, Y. (2014). Mast cells and neuroinflammation. *Med. Sci. Monit. Basic Res.* 20, 200–206. doi: 10.12659/MSMBR.893093

Dudeney, J., Sharpe, L., Jaffe, A., Jones, E. B., and Hunt, C. (2017). Anxiety in youth with asthma: a meta-analysis. *Pediatr. Pulmonol.* 52, 1121–1129. doi: 10.1002/ppul.23689

File, S. E. (2001). Factors controlling measures of anxiety and responses to novelty in the mouse. *Behav. Brain Res.* 125, 151–157. doi: 10.1016/s0166-4328(01)00292-3

Fish, E. W., Faccidomo, S., Gupta, S., and Miczek, K. A. (2004). Anxiolytic-like effects of escitalopram, citalopram, and R-citalopram in maternally separated mouse pups. *J. Pharmacol. Exp. Ther.* 308, 474–480. doi: 10.1124/jpet.103.058206

Galli, S. J., Tsai, M., and Piliponsky, A. M. (2008). The development of allergic inflammation. *Nature* 454, 445–454. doi: 10.1038/nature07204

Goodwin, R. D., Jacobi, F., and Thefeld, W. (2003). Mental disorders and asthma in the community. *Arch. Gen. Psychiatry* 60, 1125–1130. doi: 10.1001/archpsyc.60.11.1125

Guerra, S., Wright, A. L., Morgan, W. J., Sherrill, D. L., Holberg, C. J., and Martinez, F. D. (2004). Persistence of Asthma Symptoms during Adolescence: role of obesity and age at the onset of puberty. *Am. J. Respir. Crit. Care Med.* 170, 78–85. doi: 10.1164/rccm.200309-1224OC

Hahn, M. E., and Lavooy, M. J. (2005). A review of the methods of studies on infant ultrasound production and maternal retrieval in small rodents. *Behav. Genet.* 35, 31–52. doi: 10.1007/s10519-004-0854-7

Hamelmann, E., Schwarze, J., Takeda, K., Oshiba, A., Larsen, G. L., Irvin, C. G., et al. (1997). Noninvasive measurement of airway responsiveness in allergic mice using barometic plethysmography. *Am. J. Respir. Crit. Care Med.* 156, 766–775. doi: 10.1164/ajrccm.156.3.9606031

Hanania, N. A., Chapman, K. R., and Kesten, S. (1995). Adverse effects of inhaled corticosteroids. *Am. J. Med.* 98, 196–208. doi: 10.1016/S0002-9343(99)80404-5

Hogg, S. (1996). A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. *Pharmacol. Biochem. Behav.* 54, 21–30. doi: 10.1016/0091-3057(95)02126-4

Holder, M. K., and Blaustein, J. D. (2014). Puberty and adolescence as a time of vulnerability to stressors that alter neurobehavioral processes. *Front. Neuroendocrinol.* 35, 89–110. doi: 10.1016/j.yfrne.2013.10.004

Holmes, A., Yang, R. J., Lesch, K.-P., Crawley, J. N., and Murphy, D. L. (2003). Mice lacking the serotonin transporter exhibit 5-HT_{1A} receptor-mediated abnormalities in tests for anxiety-like behavior. *Neuropsychopharmacology* 28, 2077–2088. doi: 10.1038/sj.npp.1300266

Isgor, C., Kabbaj, M., Akil, H., and Watson, S. J. (2004). Delayed effects of chronic variable stress during peripubertal-juvenile period on hippocampal morphology and on cognitive and stress axis functions in rats. *Hippocampus* 14, 636–648. doi: 10.1002/hipo.10207

Julia, V., Hessel, E. M., Malherbe, L., Glaichenhaus, N., O'Garra, A., and Coffman, R. L. (2002). A restricted subset of dendritic cells captures airborne antigens and remains able to activate specific T cells long after antigen exposure. *Immunity* 16, 271–283. doi: 10.1016/s1074-7613(02)00276-5

Katon, W., Lozano, P., Russo, J., McCauley, E., Richardson, L., and Bush, T. (2007). The Prevalence of DSM-IV anxiety and depressive disorders in youth with asthma compared with controls. *J. Adolesc. Health* 41, 455–463. doi: 10.1016/j.jadohealth.2007.05.023

Lee, S. Y., Kim, J. S., Lee, J. M., Kwon, S. S., Kim, K. H., Moon, H. S., et al. (2008). Inhaled corticosteroid prevents the thickening of airway smooth muscle in murine model of chronic asthma. *Pulm. Pharmacol. Ther.* 21, 14–19. doi: 10.1016/j.pupt.2006.10.009

Lewis, S. R., Ahmed, S., Dym, C., Khaimova, E., Kest, B., and Bodnar, R. J. (2005). Inbred mouse strain survey of sucrose intake. *Physiol. Behav.* 85, 546–556. doi: 10.1016/j.physbeh.2005.06.003

Lister, R. G. (1987). The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology* 92, 180–185. doi: 10.1007/bf00177912

Lloyd, C. M. (2010). IL-33 family members and asthma—bridging innate and adaptive immune responses. *Curr. Opin. Immunol.* 22, 800–806. doi: 10.1016/j.coi.2010.10.006

Lødrup Carlsen, K. C., Mowinckel, P., Hovland, V., Håland, G., Riiser, A., and Carlsen, K. H. (2014). Lung function trajectories from birth through puberty reflect asthma phenotypes with allergic comorbidity. *J. Allergy Clin. Immunol.* 134, 917.e7–923.e7. doi: 10.1016/j.jaci.2014.05.020

McCormick, C. M., and Green, M. R. (2013). From the stressed adolescent to the anxious and depressed adult: investigations in rodent models. *Neuroscience* 249, 242–257. doi: 10.1016/j.neuroscience.2012.08.063

McCormick, C. M., Smith, C., and Mathews, I. Z. (2008). Effects of chronic social stress in adolescence on anxiety and neuroendocrine response to mild stress in male and female rats. *Behav. Brain Res.* 187, 228–238. doi: 10.1016/j.bbr.2007.09.005

McMillan, S. J., and Lloyd, C. M. (2004). Prolonged allergen challenge in mice leads to persistent airway remodelling. *Clin. Exp. Allergy* 34, 497–507. doi: 10.1111/j.1365-2222.2004.01895.x

Merkus, P. J. F. M., van Essen-Zandvliet, E. E. M., Duiverman, E. J., van Houwelingen, H. C., Kerrebijn, K. F., and Quanjer, P. H. (1993). Long-term effect of inhaled corticosteroids on growth rate in adolescents with asthma. *Pediatrics* 91, 1121–1126.

Meyer, J. H. (2007). Imaging the serotonin transporter during major depressive disorder and antidepressant treatment. *J. Psychiatry Neurosci.* 32, 86–102.

Meyer, N., Richter, S. H., Schreiber, R. S., Kloke, V., Kaiser, S., Lesch, K.-P., et al. (2016). The unexpected effects of beneficial and adverse social experiences during adolescence on anxiety and aggression and their modulation by genotype. *Front. Behav. Neurosci.* 10:97. doi: 10.3389/fnbeh.2016.00097

Molimard, M., Girodet, P.-O., Pollet, C., Fourrier-Réglat, A., Daveluy, A., Haramburu, F., et al. (2008). Inhaled corticosteroids and adrenal insufficiency: prevalence and clinical presentation. *Drug Saf.* 31, 769–774. doi: 10.2165/00002018-200831090-00005

Molnar, B. E., Buka, S. L., and Kessler, R. C. (2001). Child sexual abuse and subsequent psychopathology: results from the national comorbidity survey. *Am. J. Public Health* 91, 753–760. doi: 10.2105/ajph.91.5.753

Moretti, M. M., and Craig, S. G. (2013). Maternal versus paternal physical and emotional abuse, affect regulation and risk for depression from adolescence to early adulthood. *Child Abus. Negl.* 37, 4–13. doi: 10.1016/j.chiabu.2012.09.015

Nascimento, I., Nardi, A. E., Valença, A. M., Lopes, F. L., Mezzasalma, M. A., Nascentes, R., et al. (2002). Psychiatric disorders in asthmatic outpatients. *Psychiatry Res.* 110, 73–80. doi: 10.1016/s0165-1781(02)00029-x

Nau, F., Miller, J., Saravia, J., Ahlert, T., Yu, B., Happel, K. I., et al. (2015). Serotonin 5-HT_2 receptor activation prevents allergic asthma in a mouse model. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 308, L191–L198. doi: 10.1152/ajplung.00138.2013

Nautiyal, K. M., Dailey, C. A., Jahn, J. L., Rodriquez, E., Son, N. H., Sweedler, J. V., et al. (2012). Serotonin of mast cell origin contributes to hippocampal function. *Eur. J. Neurosci.* 36, 2347–2359. doi: 10.1111/j.1460-9568.2012.08138.x

Pasterkamp, H., Consunji-Araneta, R., Oh, Y., and Holbrow, J. (1997). Chest surface mapping of lung sounds during methacholine challenge. *Pediatr. Pulmonol.* 23, 21–30. doi: 10.1002/(sici)1099-0496(199701)23:1<21::aid-ppul3>3.0.co;2-s

Pellow, S., Chopin, P., File, S. E., and Briley, M. (1985). Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J. Neurosci. Methods* 14, 149–167. doi: 10.1016/0165-0270(85)90031-7

Porsolt, R. D., Le Pichon, M., and Jalfre, M. (1977). Depression: a new animal model sensitive to antidepressant treatments. *Nature* 266, 730–732. doi: 10.1038/266730a0

Richardson, L. P., Lozano, P., Russo, J., McCauley, E., Bush, T., and Katon, W. (2006). Asthma symptom burden: relationship to asthma severity and anxiety and depression symptoms. *Pediatrics* 118, 1042–1051. doi: 10.1542/peds.2006-0249

Romeo, R. D. (2010). Adolescence: a central event in shaping stress reactivity. *Dev. Psychobiol.* 52, 244–253. doi: 10.1002/dev.20437

Romeo, R. D. (2015). Perspectives on stress resilience and adolescent neurobehavioral function. *Neurobiol. Stress* 1, 128–133. doi: 10.1016/j.ynstr.2014.11.001

Ross, C. J. M., Davis, T. M., and Hogg, D. Y. (2007). Screening and assessing adolescent asthmatics for anxiety disorders. *Clin. Nurs. Res.* 16, 5–24. doi: 10.1177/1054773806295235

Roy, V., Belzung, C., Delarue, C., and Chapillon, P. (2001). Environmental enrichment in BALB/c mice: effects in classical tests of anxiety and exposure to a predatory odor. *Physiol. Behav.* 74, 313–320. doi: 10.1016/s0031-9384(01)00561-3

Roza, S. J., Hofstra, M. B., van der Ende, J., and Verhulst, F. C. (2003). Stable prediction of mood and anxiety disorders based on behavioral and emotional problems in childhood: a 14-year follow-up during childhood, adolescence, and young adulthood. *Am. J. Psychiatry* 160, 2116–2121. doi: 10.1176/appi.ajp.160.12.2116

Sachser, N., Hennessy, M. B., and Kaiser, S. (2011). Adaptive modulation of behavioural profiles by social stress during early phases of life and adolescence. *Neurosci. Biobehav. Rev.* 35, 1518–1533. doi: 10.1016/j.neubiorev.2010.09.002

Sadler, A. M., and Bailey, S. J. (2016). Repeated daily restraint stress induces adaptive behavioural changes in both adult and juvenile mice. *Physiol. Behav.* 167, 313–323. doi: 10.1016/j.physbeh.2016.09.014

Saglani, S., Mathie, S. A., Gregory, L. G., Bell, M. J., Bush, A., and Lloyd, C. M. (2009). Pathophysiological features of asthma develop in parallel in house dust mite-exposed neonatal mice. *Am. J. Respir. Cell Mol. Biol.* 41, 281–289. doi: 10.1165/rcmb.2008-0396oc

Salmond, R. J., Mirchandani, A. S., Besnard, A. G., Bain, C. C., Thomson, N. C., and Liew, F. Y. (2012). IL-33 induces innate lymphoid cell-mediated airway inflammation by activating mammalian target of rapamycin. *J. Allergy Clin. Immunol.* 130, 1159.e6–1166.e6. doi: 10.1016/j.jaci.2012.05.018

Schröder, P. C., Casaca, V. I., Illi, S., Schieck, M., Michel, S., Böck, A., et al. (2016). IL-33 polymorphisms are associated with increased risk of hay fever and reduced regulatory T cells in a birth cohort. *Pediatr. Allergy Immunol.* 27, 687–695. doi: 10.1111/pai.12597

Shajib, M. S., and Khan, W. I. (2015). The role of serotonin and its receptors in activation of immune responses and inflammation. *Acta Physiol.* 213, 561–574. doi: 10.1111/apha.12430

Sjöberg, L. C., Nilsson, A. Z., Lei, Y., Gregory, J. A., Adner, M., and Nilsson, G. P. (2017). Interleukin 33 exacerbates antigen driven airway hyperresponsiveness, inflammation and remodeling in a mouse model of asthma. *Sci. Rep.* 7:4219. doi: 10.1038/s41598-017-03674-0

Skobeloff, E. M., Spivey, W. H., St Clair, S. S., and Schoffstall, J. M. (1992). The influence of age and sex on asthma admissions. *JAMA* 268, 3437–3440. doi: 10.1001/jama.1992.03490240045034

Smith, T. F., and Hudgel, D. W. (1980). Decreased ventilation response to hypoxia in children with asthma. *J. Pediatr.* 97, 736–741. doi: 10.1016/s0022-3476(80)80255-1

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neurosci. Biobehav. Rev.* 24, 417–463. doi: 10.1016/s0149-7634(00)00014-2

Strekalova, T., Spanagel, R., Bartsch, D., Henn, F. A., and Gass, P. (2004). Stress-induced anhedonia in mice is associated with deficits in forced swimming and exploration. *Neuropsychopharmacology* 29, 2007–2017. doi: 10.1038/sj.npp.1300532

Tai, A., Tran, H., Roberts, M., Clarke, N., Gibson, A. M., Vidmar, S., et al. (2014a). Outcomes of childhood asthma to the age of 50 years. *J. Allergy Clin. Immunol.* 133, 1572.e3–1578.e3. doi: 10.1016/j.jaci.2013.12.1033

Tai, A., Tran, H., Roberts, M., Clarke, N., Wilson, J., and Robertson, C. F. (2014b). The association between childhood asthma and adult chronic obstructive pulmonary disease. *Thorax* 69, 805–810. doi: 10.1136/thoraxjnl-2013-204815

Takahashi, A., Yap, J. J., Bohager, D. Z., Faccidomo, S., Clayton, T., Cook, J. M., et al. (2009). Glutamatergic and GABAergic modulations of ultrasonic vocalizations during maternal separation distress in mouse pups. *Psychopharmacology* 204, 61–71. doi: 10.1007/s00213-008-1437-8

Tanaka, H., Masuda, T., Tokuoka, S., Takahashi, Y., Komai, M., Nagao, K., et al. (2002). Time course study on the development of allergen-induced airway remodeling in mice: the effect of allergen avoidance on established airway remodeling. *Inflamm. Res.* 51, 307–316. doi: 10.1007/pl00000309

Temelkovski, J., Hogan, S. P., Shepherd, D. P., Foster, P. S., and Kumar, R. K. (1998). An improved murine model of asthma: selective airway inflammation, epithelial lesions and increased methacholine responsiveness following chronic exposure to aerosolised allergen. *Thorax* 53, 849–856. doi: 10.1136/thx.53.10.849

Theoharides, T. C., Alysandratos, K. D., Angelidou, A., Delivanis, D. A., Sismanopoulos, N., Zhang, B., et al. (2012). Mast cells and inflammation. *Biochim. Biophys. Acta* 1822, 21–33. doi: 10.1016/j.bbadis.2010.12.014

Tirelli, E., Laviola, G., and Adriani, W. (2003). Ontogenesis of behavioral sensitization and conditioned place preference induced by psychostimulants in laboratory rodents. *Neurosci. Biobehav. Rev.* 27, 163–178. doi: 10.1016/s0149-7634(03)00018-6

Tonelli, L. H., Katz, M., Kovacsics, C. E., Gould, T. D., Joppy, B., Hoshino, A., et al. (2009). Allergic rhinitis induces anxiety-like behavior and altered social interaction in rodents. *Brain Behav. Immun.* 23, 784–793. doi: 10.1016/j.bbi.2009.02.017

Whaley, S. E., Pinto, A., and Sigman, M. (1999). Characterizing interactions between anxious mothers and their children. *J. Consult. Clin. Psychol.* 67, 826–836. doi: 10.1037/0022-006x.67.6.826

Yee, M., Chess, P. R., McGrath-Morrow, S. A., Wang, Z., Gelein, R., Zhou, R., et al. (2009). Neonatal oxygen adversely affects lung function in adult mice without altering surfactant composition or activity. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 297, L641–L649. doi: 10.1152/ajplung.00023.2009

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
published: 08 May 2018
doi: 10.3389/fnbeh.2018.00091

Post-earthquake Distress and Development of Emotional Expertise in Young Adults

Francesca Pistoia[1], Massimiliano Conson[2], Antonio Carolei[1], Maria G. Dema[3], Alessandra Splendiani[3], Giuseppe Curcio[3] and Simona Sacco[1]*

[1]Department of Biotechnological and Applied Clinical Sciences, Neurological Institute, University of L'Aquila, L'Aquila, Italy, [2]Neuropsychology Laboratory, Department of Psychology, University of Campania Luigi Vanvitelli, Caserta, Italy, [3]Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy

After a natural disaster like an earthquake about 15% of the population experience a post-traumatic stress disorder (PTSD). However, even those without a diagnosis of PTSD can suffer from disorders of the affective sphere, including anxiety, depression and alteration of emotion recognition. The objective of this study was to investigate the neuropsychological and emotional profile of students living in the earthquake-affected areas of L'Aquila, Italy. A group of students living in L'Aquila at the time of the 2009 earthquake was recruited, and compared to a control group of students not living in any earthquake-affected areas. Participants were assessed by means of the Beck Depression Inventory (BDI) scale, the State-Trait Anxiety Inventory (STAI), the Insomnia Severity Index (ISI), the Intolerance of Uncertainty Scale Short Form, the Uncertainty Response Scale (URS), the Anxiety Sensitivity Index 3 (ASI-3), and the Eysenck Personality Questionnaire-Revised Short Form (EPQ-RS). Participants also took part in two behavioral experiments aimed at evaluating their ability to recognize facial expressions (by means of the Ekman and Friesen Pictures of Facial Affect) and to evaluate emotionally evocative scenes (by means of the International Affective Picture System (IAPS)). Results showed that students living in the earthquake-affected areas had a general increase of anxiety and anticipation of threats. Moreover, students living in the earthquake-affected areas showed a significantly higher overall accuracy in recognizing facial expressions as compared to controls. No significant differences between the two groups were detected in the evaluation of emotionally evocative scenes. The novel result lies in the greater accuracy of earthquake victims in recognizing facial expressions, despite the lack of differences from controls in evaluating affective evocative scenes. The trauma exposure may have increased vigilance for threats in earthquake victims, leading them to systematically pay attention to potential signs of approaching threats, such as emotional facial expressions, thus progressively developing particular "emotional expertise."

Keywords: earthquake, anxiety, depression, emotional, expertise

OPEN ACCESS

Edited by:
Erica R. Glasper, University of Maryland, College Park, United States

Reviewed by:
Jennifer Strafford Stevens, Emory University School of Medicine, United States
Gianluca Serafini, Dipartimento di Neuroscienze e Organi di Senso, Ospedale San Martino (IRCCS), Italy

****Correspondence:***
Giuseppe Curcio
giuseppe.curcio@univaq.it

Received: *08 March 2018*
Accepted: *23 April 2018*
Published: *08 May 2018*

Citation:
Pistoia F, Conson M, Carolei A, Dema MG, Splendiani A, Curcio G and Sacco S (2018) Post-earthquake Distress and Development of Emotional Expertise in Young Adults. Front. Behav. Neurosci. 12:91. doi: 10.3389/fnbeh.2018.00091

INTRODUCTION

After a natural disaster, like an earthquake, population has an increased vulnerability to developing psychological and psychiatric disorders. The most frequently reported is Post-Traumatic Stress Disorder (PTSD), which is a mental health problem triggered by life-threatening events (Neria et al., 2008). The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) criteria for PTSD include the direct or indirect exposure to a terrifying event; the occurrence of at least one of five intrusion symptoms; the presence of an avoidance behavior concerning trauma-related stimuli and of negative alterations in cognitions and mood; and the occurrence of trauma-related alterations in arousal and reactivity. Moreover, in order to fulfill criteria for the PTSD diagnosis, symptoms are required to last for more than 1 month, to create distress or functional impairment in person affected, and to not be attributable to other illnesses or to the use of medications or substances (American Psychiatric Association, 2013). Prevalence rates of PTSD following an earthquake are extremely heterogeneous across studies and range from 4% to 67% (Tang et al., 2017). Factors accounting for this variability include the population studied, the age groups considered, the time elapsed since the traumatic event, the sample size and the study design. As mental health looks like a continuum, it is reasonable to expect that a population which has been exposed to a natural disaster cannot simply be split into two groups, one suffering from well-defined psychiatric disorders and the other enjoying optimal mental health. It is likely that a certain proportion of individuals belong to a gray zone where a psychiatric illness is not diagnosed, but the person is not experiencing complete mental well-being. This is to be expected even more in the young, in whom traumatic experiences may exert a profound impact on psychological and emotional behaviors. Such an impact may not necessarily manifest as impaired processing of emotions, but might rather lead to heightened sensitivity to specific emotional signals, especially those conveying self-relevant potential threatening information, as in the case of negative emotional facial expressions (Bell et al., 2017).

Defining the specific nature of these "compensative" emotional responses could have relevant translational implications, including the implementation of interventions aimed at encouraging the development of coping strategies after natural disasters. International guidelines recommend using cognitive-behavioral therapy (CBT) a few weeks after a disaster or other shocking events to reduce psychopathological symptoms, in particular those related to PTSD (Te Brake et al., 2009). A recent review of the literature has demonstrated that, among the cognitive-behavior therapy techniques, exposure techniques seem particularly effective for treating PTSD after earthquakes (Lopes et al., 2014). However, recent cognitive models of anxiety disorders consistently reported attentional biases toward threat-related stimuli not only in persons with a clinical condition but also in nonclinical individuals reporting high levels of anxiety (Dalgleish et al., 2003; Bar-Haim et al., 2007; MacLeod and Grafton, 2016). It is worth noting here, that together with PTSD, depression is one of the most common psychiatric disorders appearing in earthquake survivors several weeks or months after traumatic events, and often persisting for years (Yule, 2001). Interestingly, current views on depression underlined the central role of cognitive dysfunctions (Gonda et al., 2015), and especially of attentional biases, in the etiology and maintenance of the disorder (Disner et al., 2011). On this basis, clarifying the nature of the dysfunctional emotional processing in earthquake-exposed persons could allow choosing the most effective cognitive-behavioral technique allowing survivors to boost resilience and to acquire specific mental skills to manage threats.

In this venue, the objective of the present study was to investigate the neuropsychological and emotional profile of earthquake-exposed university students and to identify any specificity in the way they process affective information including emotional facial expressions and emotionally evocative scenes. Moving from previous evidence demonstrating an increased sensitivity to negative facial emotions in earthquake-exposed individuals as compared to non-exposed controls (Bell et al., 2017), here we aimed at assessing whether this enhanced response to visual affective stimuli was specific to facial expressions or, instead, it was related to a pervasive enhanced sensitivity towards all visual stimuli conveying affective information, as in the case of affective complex scenes. Thus, we compared earthquake-exposed students with non-exposed control students on recognition of facial expressions (by means of the Ekman and Friesen's set of pictures) and on judgment of emotionally evocative scenes (by means of the International Affective Picture System (IAPS)).

Basing on previous literature reports, the following alternative findings may be expected: (i) earthquake-exposed and not exposed students may show a similar emotional profile without significant differences in processing emotional stimuli, whether these be facial expression or emotional evocative scenes; (ii) earthquake-exposed students may have developed an increased and generalized sensitivity to emotional information, resulting in increased accuracy in processing all kinds of emotional information, both emotional facial expressions and emotionally evocative scenes; and (iii) earthquake-exposed students may show a selective increased accuracy in recognizing emotional facial expression, despite an unchanged perception of their own internal emotional responses to evocative scenes not involving faces. The results of the present study might provide empirical evidence about the engagement of specific coping and resilience strategies in young adults after an environmental traumatic event.

MATERIALS AND METHODS

Experimental Setting

The earthquake epicenter of L'Aquila (central Italy) was used as the experimental setting. On April 6th 2009, L'Aquila was hit by an earthquake lasting 20 s and with a magnitude of 6.3 on the moment magnitude scale. The earthquake caused 309 deaths and the destruction of the city, with 65,000 inhabitants being forced to leave their homes. The main earthquake was followed by thousands of aftershocks in the subsequent months

with a severe psychological burden on the whole population. About 7 years after this devastating earthquake, with the city only partially rebuilt, the population experienced additional earthquakes (August and October 2016 and January 2017). Although not causing further destruction, these resulted in great psychological distress for the people of L'Aquila, who had not yet recovered from the psychological, social and economic consequences of the 2009 earthquake.

Participants

A sample of students living in L'Aquila at the time of the 2009 earthquake was recruited for the participation in the study. To be included, participants had to fulfill the following criteria: age >18 years at the time of the inclusion; stable residence in L'Aquila at the time of the 2009 earthquake; no history of previous or coexistent neurological or psychiatric diseases, including PTSD, or assumption of drugs or substances acting on the central nervous system; and signed informed consent to participate in the study. A control group of students, matched by age and sex, not living in any earthquake-affected areas, was recruited and used for comparison: these participants were all psychologically healthy individuals without a personal or family history of mental illness. The whole sample included 107 students. Forty-eight subjects belonged to the experimental earthquake-exposed group (20 males and 28 females, mean age = 22.6, SD = 2.3 years) and 59 to the control group (30 males and 29 females, mean age = 23.1, SD = 1.6 years).

The research protocol was approved by the Internal Review Board of the University of L'Aquila (01/2017). The study was conducted in accordance with the ethical standards of the Helsinki Declaration and signed informed consent was obtained from all the participants.

Procedures

Self-Reported Measures

All participants were assessed by means of the following formalized measures: the Beck Depression Inventory (BDI; Beck, 1967), the State-Trait Anxiety Inventory (STAI; Spielberger et al., 1983; Pedrabissi and Santiniello, 1989), the Insomnia Severity Index (ISI; Bastien et al., 2001; Castronovo et al., 2016), the Intolerance of Uncertainty Scale Short Form (IUS-12; Freeston et al., 1994), the Uncertainty Response Scale (URS; Greco and Roger, 2001), the Anxiety Sensitivity Index 3 (ASI-3; Taylor et al., 2007; Petrocchi et al., 2015) and the Eysenck Personality Questionnaire-Revised Short Form (EPQ-RS; Eysenck et al., 1985; Picconi et al., 2018).

The BDI (Beck, 1967) is a 21 items self-report inventory; it is one of the most widely used psychometric tests for the assessment of depression severity. Total score can range from 0 to 63, with higher scores indicating increasing level of depressive symptoms. The total score is usually used as dependent variable.

The STAI (Spielberger et al., 1983; Pedrabissi and Santiniello, 1989) is a commonly used measure of trait and state anxiety: here, the 20 items only for the assessment of trait anxiety have been used. Total score can range between 20 and 60, and also in this case high score reflect high level of anxiety. The total score has been considered as dependent variable.

The ISI (Bastien et al., 2001; Castronovo et al., 2016) is a 7-item self-report questionnaire assessing the nature, severity and impact of insomnia. The usual recall period is the "last month." This tool allows evaluating different dimensions (sleep onset, sleep maintenance and early morning awakening problems, sleep dissatisfaction, interference with daytime functioning, noticeability of sleep problems by others, and distress caused by the sleep difficulties). A Likert scale is used to rate each item, yielding a total score ranging from 0 to 28, with higher scores indicating higher severity of insomnia symptoms. The total score has been taken into consideration as dependent variable.

The Tolerance of Uncertainty Scale Short Form (IUS-12; Freeston et al., 1994) is a short version of the original 27-item Intolerance of Uncertainty Scale that measures responses to uncertainty, ambiguous situations and the future. The 12 items are rated on a 5-point Likert scale and can provide a measure of both Prospective Anxiety and Inhibitory Anxiety, as well as a total measure of uncertainty (by summing the scores to all the 12 items). We considered the total score as dependent variable.

The URS (Greco and Roger, 2001) is a scale for the evaluation of styles of coping with uncertainty. The 48 items are rated on a 4-point Likert scale and can provide a measure of three subscales (Emotional Uncertainty, Desire for Control, Cognitive Uncertainty). As dependent variable, we considered both the three subscales scores and the total score.

The ASI-3 (Taylor et al., 2007; Petrocchi et al., 2015) is an 18-item, self-report measure developed to assess vulnerability to anxiety. Each item is rated on a 5-point Likert scale and higher scores reflect high level of anxiety. As dependent variables, we considered the Physical Concerns, Social Concerns and Cognitive Concerns subscales as well as the total score (sum of all the three subscales).

Finally, the EPQ-RS (Eysenck et al., 1985; Picconi et al., 2018) was used to assess personality characteristics of participants. EPQ-RS is a self-reported questionnaire with 48 dichotomous (yes, no) items, 12 for each of the traits of neuroticism, extraversion/intraversion and psychoticism, and 12 for the lie scale. As dependent variables, the scores of neuroticism, extraversion/intraversion, and psychoticism scales have been taken into consideration.

Experimental Tasks

Task 1. Recognition of Facial Expressions

Photographs of 10 actors (five males, five females) were taken from the Ekman and Friesen set of Pictures of Facial Affect (Ekman and Friesen, 1976; Ekman, 1993). Each model posed facial expressions corresponding to six basic emotions: happiness, sadness, anger, fear, disgust and surprise. The complete image set therefore included 60 stimuli (10 faces × 6 emotions). For each stimulus, subjects were required to name the expressed emotion selecting from six labels (happiness, sadness, anger, fear, disgust or surprise), and then to rate the emotion intensity expressed in the picture on a scale of 1–9 (1 = none, 5 = moderate, 9 = extreme).

Task 2. Evaluation of Emotionally Evocative Scenes

Stimuli consisted of complex pictures selected from the IAPS (Lang et al., 1997; Bradley and Lang, 2007). Stimulus selection was based on the results of a pilot study in which 80 university students assigned 200 IAPS scenes to one of six emotion labels (i.e., happiness, sadness, anger, fear, disgust and surprise) defined on the basis of emotional category data on IAPS (Lang et al., 1993; Davis et al., 1995; Bradley et al., 2001; Mikels et al., 2005). Different studies demonstrated that many of the IAPS images elicit multiple discrete emotions (Bradley et al., 2001; Mikels et al., 2005). For this reason, in the present experiment (see also Pistoia et al., 2010, 2015), we only used images classified by at least 70% of normal subjects consistently within one single emotional category. On this basis, we had to exclude stimuli intended to elicit surprise, because no item of this category reached the defined consistency level. Moreover, following Mikels et al.'s (2005) approach, we also excluded images being classified within two or more emotional categories. Therefore, the resulting image set included 30 images (6 items × 5 emotions), each consistently eliciting a single emotional category among the following emotions (Lang et al., 1993; Davis et al., 1995; Bradley et al., 2001; Mikels et al., 2005): happiness (scenes involving babies or sporting events), sadness (scenes of illness, cemeteries or funeral scenes), anger (scenes of human violence), fear (scenes of snakes or spiders), and disgust (scenes of rubbish or rats). Each stimulus was presented twice for a total of 60 items.

The subjects were first required to provide an emotional category rating by choosing among the five categories (happiness, sadness, anger, fear, or disgust) the one corresponding to the subjectively evoked emotion; the response was scored 1 if the subject selected the emotional category consistently evocated by the image, otherwise the response was scored 0. Then, the subjects had to rate how strong their own emotional response was on a scale of 1–9 (1 = not at all, 5 = moderately, 9 = extremely).

Statistical Analysis

For the self-reported measures, a multivariate analysis of variance was performed with group and sex as independent variables and with scores on the self-reported measures as dependent variables. Level for statistical significance was set at $p < 0.0033$ according to Bonferroni's correction for multiple comparisons. For the behavioral measures (recognition of facial expressions and evaluation of emotionally evocative scenes) a three-way mixed analysis of variance (ANOVA) was performed, with emotion as a within-subject factor and with group and sex as between-subject factors.

RESULTS

Self-Reported Measures

The multivariate results showed significant effects for group (Pillai's Trace = 0.241; Wilks' Lambda = 0.759; $F_{(15,89)} = 1.885$; $p = 0.035$, partial eta squared = 0.241) and for sex (Pillai's Trace = 0.313; Wilks' Lambda = 0.687; $F_{(15,89)} = 2.698$; $p = 0.45$, partial eta squared = 0.230). The interaction between group and sex was not significant ($p > 0.05$).

TABLE 1 | Scores (mean and SD) of the two groups on the self-reported measures.

	Controls		Earthquake victims	
Measures	**Mean**	**SD**	**Mean**	**SD**
ISI	5.05	3.5	6.35	4
IUS-12	35.00	15.2	42.54	16.7
URS total score*	121.03	12.1	128.56	13.3
URS emotional uncertainty*	26.53	6.1	31.69	7.4
URS desire for control	47.22	7.2	46.13	7.7
URS cognitive uncertainty	47.41	8.5	51.10	8.9
STAI*	37.63	8.2	42.46	7.8
BDI	6.54	5.2	8.56	5.8
ASI-3 total score*	10.90	8.3	18.21	13.3
ASI-3 physical concerns	3.41	3.9	5.46	5.6
ASI-3 cognitive concerns*	4.05	3.6	6.77	5.1
ASI-3 social concerns*	3.44	3.4	6.25	5.1
EPQ-R Extraversion/Introversion	9.05	2.8	8.48	3.2
EPQ-R neuroticism*	4.46	2.7	6.33	3.3
EPQ-R psychoticism	3.22	1.9	2.67	1.5

*ISI, Insomnia Severity Index; IUS-12, Tolerance of Uncertainty Scale Short Form; URS, Uncertainty Response Scale; STAI, State-Trait Anxiety Inventory; BDI, Beck Depression Inventory; ASI-3, Anxiety Sensitivity Index; EPQ-R, Eysenck Personality Questionnaire-Revised Short Form. *Significant at $p \leq 0.003$.*

For group (scores of the two groups on all the measures are reported in **Table 1**), there were significant univariate effects for: URS Total score ($F_{(1,103)} = 9.079$, $p = 0.003$, partial eta squared = 0.081), URS Emotional Uncertainty ($F_{(1,103)} = 15.399$, $p = 0.0001$, partial eta squared = 0.130), STAI-2 ($F_{(1,103)} = 10.036$, $p = 0.002$, partial eta squared = 0.089), ASI Total Score ($F_{(1,103)} = 12.740$, $p = 0.001$, partial eta squared = 0.110), ASI Cognitive Concerns ($F_{(1,103)} = 10.944$, $p = 0.001$, partial eta squared = 0.096), ASI Social Concerns ($F_{(1,103)} = 11.777$, $p = 0.0001$, partial eta squared = 0.118), and EPQ Neuroticism Scale ($F_{(1,103)} = 10.260$, $p = 0.002$, partial eta squared = 0.091). No other difference was significant at the Bonferroni corrected p value.

For sex, there was only a significant univariate effect for IUS-12 ($F_{(1,103)} = 15.322$, $p = 0.0001$, partial eta squared = 0.129), with females scoring higher (mean = 44, SD = 16.1) than males (mean = 32.6, SD = 18.2). No other univariate effect was significant at the Bonferroni corrected p value.

Experimental Tasks

Recognition of Facial Expressions

Percentages of correct responses are shown in **Table 2**. A three-way mixed ANOVA was carried out, with emotion (disgust, happiness, fear, anger, surprise and sadness) as a within-subject factor and with group and sex as between-subject factors. This showed a significant main effect of emotion ($F_{(5,515)} = 36.897$, $p = 0.0001$, partial eta squared = 0.264), with recognition of fear (0.60) being worse than all other emotions (disgust = 0.86; happiness = 0.99; anger = 0.89; surprise = 0.96; and sadness = 0.79). Results also showed significant main effects of group ($F_{(1,103)} = 16.832$, $p = 0.0001$, partial eta squared = 0.140), with overall accuracy being higher in earthquake victims (mean = 0.89, SD = 0.14) than in controls (mean = 0.81, SD = 0.12), and of sex ($F_{(1,103)} = 4.208$,

TABLE 2 | Accuracy (mean and SD) and intensity rating on correctly recognized emotions (mean and SD) of the two groups on recognition of facial expressions task.

	Controls		Earthquake victims	
Accuracy	**Mean**	**SD**	**Mean**	**SD**
Disgust	0.84	0.2	0.88	0.3
Happiness	0.98	0.1	1.00	0
Fear	0.59	0.3	0.61	0.5
Anger	0.079	0.2	0.98	0.1
Surprise	0.93	0.2	0.99	0.2
Sadness	0.73	0.3	0.86	0.3
Intensity rating				
Disgust	6.20	1.1	6.59	0.9
Happiness	6.61	0.86	6.83	0.8
Fear	6.24	1.3	6.60	0.9
Anger	5.66	1.4	5.78	1.2
Surprise	6.38	0.9	6.55	1.1
Sadness	5.57	1.2	5.63	1.3

TABLE 3 | Emotional category rating (mean and SD) and intensity rating of affective scenes (mean and SD) of the two groups on judgment of emotionally evocative scenes task.

	Controls		Earthquake victims	
	Mean	**SD**	**Mean**	**SD**
Emotional category rating				
Disgust	0.80	0.2	0.77	0.2
Happiness	0.91	0.3	0.90	0.2
Fear	0.73	0.2	0.73	0.3
Anger	0.62	0.3	0.56	0.3
Sadness	0.87	0.4	0.91	0.1
Intensity rating				
Disgust	6.05	1.5	6.06	1.6
Happiness	6.46	1.4	6.87	1.4
Fear	6.16	1.8	6.43	1.4
Anger	7.44	1.3	7.79	1.3
Sadness	6.27	1.6	6.35	1.4

$p = 0.043$, partial eta squared = 0.039), with females (mean = 0.87, SD = 0.13) being more accurate than males (mean = 0.83, SD = 0.14). No interaction was significant (all $p > 0.05$). *Post hoc* Bonferroni's pairwise comparisons on the main effect of emotion demonstrated that recognition of happiness was significantly easier than all other emotions (all $p < 0.001$), followed by surprise ($p = 0.01$ vs. remaining emotions). No significant differences were detected between recognition of anger, sadness, or disgust (all $p = 0.05$), whereas recognition of fear was significantly worse than all other emotions ($p = 0.005$).

For intensity rating on correctly recognized emotions, we performed the same ANOVA as above, and results showed a significant main effect of emotion ($F_{(5,515)} = 37.942$, $p = 0.0001$, partial eta squared = 0.271), with happy faces (6.72) being rated more intense than all other emotion expressions (surprise = 6.45, fear = 6.41, disgust = 6.40, anger = 5.71, and sadness = 5.60). Results also showed a marginally significant main effect of sex ($F_{(1,103)} = 3.939$, $p = 0.050$, partial eta squared = 0.037), with females' rating (mean = 6.38, SD = 0.11) being higher than males' (mean = 6.05, SD = 0.11), whereas the main effect of group was not significant ($p > 0.05$). No interaction was significant (all $p > 0.05$). *Post hoc* Bonferroni's pairwise comparisons on the main effect of emotion showed that rating of emotional intensity was significantly higher for happiness with respect to all the other emotions ($p = 0.030$), followed by surprise, fear and disgust which were rated significantly more intense than anger and sadness ($p = 0.0001$). Ratings of surprise, fear and disgust did not significantly differ from each other ($p > 0.05$).

Evaluation of Emotionally Evocative Scenes

Emotional category ratings are shown in **Table 3**. A three-way mixed ANOVA was carried out, with emotion (disgust, happiness, fear, anger and sadness) as a within-subject factor and group as a between-subject factor. This did not reveal significant main effects of group or sex (both $p > 0.05$) but showed a significant main effect of emotion ($F_{(4,412)} = 39.545$, $p = 0.0001$, partial eta squared = 0.277), with the rating of scenes evocating anger (0.59) being more difficult as compared to the other emotions (fear = 0.73; disgust = 0.78; sadness = 0.89; happiness = 0.90). *Post hoc* Bonferroni's pairwise comparisons demonstrated that the rating of scenes evocating anger was significantly more difficult than that of the other emotions (all $p < 0.005$). No significant differences were detected between the rating of scenes evocating anger and disgust and between scenes eliciting sadness and happiness (all $p > 0.05$); the rating of scenes eliciting sadness and happiness was significantly easier that that concerning the other emotions (all $p < 0.0001$).

Intensity ratings on correct responses were analyzed by the same three-way mixed ANOVA as above, and results showed a significant main effect of sex ($F_{(1,103)} = 10.362$, $p = 0.002$, partial eta squared = 0.091), with females' rating (mean = 6.89, SD = 0.14) being more higher than males' (mean = 6.24, SD = 0.15), whereas the main effect of group was not significant ($p > 0.05$). Moreover, results showed a significant effect of emotion ($F_{(4,412)} = 37.678$, $p = 0.0001$, partial eta squared = 0.229), with scenes evocating anger (7.56) being rated as more intense than all other scenes (happiness = 6.65, fear = 6.30, sadness = 6.30, and disgust = 6.03). *Post hoc* Bonferroni's pairwise comparisons on the main effect of emotion showed that the rating of emotional intensity was significantly higher for scenes evocating anger with respect to all the other scenes ($p = 0.0001$), whereas the ratings of scenes evocating happiness, fear, sadness and disgust did not significantly differ between each other ($p > 0.05$).

DISCUSSION

The main findings of the present study demonstrated a general increase of anxiety and anticipation of threats, as well as a tendency toward sleep problems in earthquake victims, which is consistent with previous literature on the mental health and psychological problems of earthquake victims (Maltais et al., 2001; Tempesta et al., 2013; Ferrara et al., 2016; Bianchini et al., 2017; Labra et al., 2017). Importantly, moreover, behavioral experiments demonstrated significantly higher accuracy of the earthquake-exposed group in recognizing facial expressions as compared to the control group. This was notwithstanding a comparable capacity to evaluate own emotional response to affective scenes. Considering this combination of results,

we would suggest that exposure to earthquake selectively increased vigilance for threat detection leading earthquake victims to systematically pay attention to stimuli signaling potential threats, as in the case of emotional facial expressions. Our interpretation is consistent with that recently provided by Bell et al. (2017), who studied individuals exposed to the 2010–2011 Canterbury (new Zealand) earthquake. They found that both individuals with PTSD and earthquake-exposed individuals without PTSD had increased accuracy in recognition of emotional facial expressions as compared to a non-exposed control group. The authors suggested that the earthquake-exposure affected the recognition of facial expressions, independently from the development of a clinical psychopathological disease, by increasing the sensitivity to threat-related stimuli.

Hypervigilance towards environmental stimuli, which signal potential sources of threat, can be an adaptive mechanism following the exposure to a traumatic event as it can be advantageous to efficiently check the surrounding context in order to detect an upcoming threatening event. In this respect, being accurate in processing the others' facial expressions may be particularly useful, because such stimuli provide highly relevant social information and play a key role in emotional appraisal of self-relevant threats. However, it is worth noting that the increased accuracy of earthquake-exposed participants was not restricted to threat-related expressions (such as angry and fearful faces). This result is again consistent with the findings of Bell et al. (2017) who suggested that this trend could be related to the prolonged exposure to aftershocks in the earthquake-exposed individuals. As an alternative explanation, we suggest that the development of a hypersensitivity to emotional facial expressions, irrespective of the specific emotional category, could represent an effective way to rapidly detect the presence of self-relevant threatening events in the surroundings (Sander et al., 2003). Disentangling between these two alternative interpretations was outside the main aims of the present study, but this issue merits a direct investigation.

The novel result of the present study was that the higher accuracy in emotional faces recognition was a specific emotional response to the traumatic event rather than the expression of a general, heightened sensitivity to affective information. This was demonstrated by the findings that earthquake victims did not show any difference from non-exposed participants in evaluating the nature of their own reaction to the presentation of affective scenes not involving faces. A dissociation between explicit recognition of emotional facial expressions and evaluation of affective scenes has been previously reported by our group in studies on clinical populations with specific neurological disorders involving damage of motor and of sensory pathways, respectively (Pistoia et al., 2010, 2015). The present study could demonstrate for the first time that such a selective effect on processing of emotional faces can also be the result of exposure to a stressful, traumatic event and can be related to the development of specific expertise allowing earthquake victims to effectively detect threats in the surrounding environment.

Bianchini et al. (2017) investigated the relationship between the presence of anxiety and depressive symptoms following the trauma and the implementation of coping strategies (post-traumatic growth) within a university student community exposed to the 2009 L'Aquila earthquake, 2 years after the traumatic experience. Results demonstrated that 13.3% of the sample reported anxiety and about 60% showed variable levels of depression, with moderate levels of depression being predictive of post-traumatic growth. The authors suggested that some psychopathological conditions with a typically negative connotation, such as depression, might promote the development of a positive post-traumatic response, likely through the implementation of metacognitive skills that, in turn, can favor positive and functional coping strategies. Within this interpretative framework, greater accuracy in recognizing facial expressions could represent a "positive" emotional response of earthquake victims, who are forced to constantly deal with emotional signals of threat. In recent years a number of behavioral and neuroimaging studies have demonstrated that experience can shape the persons' ability to analyze and respond to specific categories of stimuli. For instance, while observing needles being inserted into others' body parts, physicians who are expert in acupuncture showed a specific brain activation involving areas devoted to the understanding of others' mind and to the regulation of affective responses (Cheng et al., 2007). Accordingly, Conson et al. (2013) demonstrated that professional actors with specific training in voluntary activation of mimicry to reproduce character's emotions were better at the explicit recognition of facial expressions than both non-professionals and professional actors trained to infer other's inner states from reading the emotional context. The authors argued that experience can selectively influence explicit recognition of others' facial expressions, depending on the kind of "emotional expertise" acquired. The present study suggests that such expertise in explicitly decoding others' facial expressions can be the result of trauma exposure. However, it seems to be a maladaptive rather than a functional emotional response to trauma, since the earthquake victims showed a higher degree of anxiety, insomnia and threat anticipation. The present results can therefore be best accounted for by a hypervigilance toward threat-related stimuli, as consistently found not only in people affected by different anxiety disorders (e.g., generalized anxiety disorder, specific phobias, social phobia or PTSD), but also in nonclinical individuals reporting high levels of anxiety (Dalgleish et al., 2003; Bar-Haim et al., 2007). Indeed, although hypervigilance to threats facilitates the detection of danger in the environment and helps the organism to respond effectively to threatening situations, it plays a central role in the etiology and maintenance of anxiety disorders (Beck, 1976; Eysenck, 1992; Mathews and MacLeod, 2002).

One possible limitation of the study lies in the fact that we tested the processing of emotional faces by employing highly prototypical stimuli displaying emotional faces with a straight gaze, as previously made in most of the studies addressing the same issue. However, one should take into account that such a laboratory setting cannot completely simulate the action of

processing emotional facial expressions in real-life situations (Hess and Blairy, 2001). In this respect, one could manipulate both emotional category and direction of eye gaze (direct vs. averted) of the facial expression, thus developing more ecologically valid, self-relevant threatening stimuli (Sander et al., 2003; Ponari et al., 2013).

From a translational point of view, the present findings could pave the way for the implementation of specific preventive and treatment options for earthquake-exposed people by exploiting available techniques of cognitive biases modification, such as attentional bias modification (MacLeod and Clarke, 2013). Attentional bias modification is an emerging treatment approach designed to modify the patterns of attentional selectivity favoring the processing of threatening information. Importantly here, several studies with both clinical and non-clinical populations have demonstrated that this technique can reduce emotional vulnerability (Clarke et al., 2014). We did not use a classical attentional bias task (MacLeod et al., 1986; Bar-Haim et al., 2007) to demonstrate a condition of hypervigilance toward emotional faces in earthquake victims, but our findings suggest that the implementation of an attentional bias modification paradigm might help to reduce the pattern of anxiety responses in earthquake-exposed people. It might also prevent the possible development of clear psychopathological disorders in victims showing subclinical conditions.

In conclusion, the present study indicates a greater accuracy of earthquake victims in recognizing facial expressions, despite the lack of difference from controls in evaluating emotionally evocative scenes. A possible explanation for this effect is that trauma exposure increases threat detection in earthquake victims, leading them to systematically pay much more attention to every kind of potential sign of threat. This may lead people exposed to trauma to progressively develop specific, "emotional expertise." Further studies are necessary to confirm our findings and to implement preventive and treatment approaches to boost resilience and encourage coping strategies in exposed populations.

AUTHOR CONTRIBUTIONS

FP, MC, AC, MGD, AS, GC and SS provided their substantial contributions to the conception of the work, the acquisition, analysis and interpretation of data and to the writing of the draft manuscript.

REFERENCES

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders.* 5th Edn. Washington, DC: American Psychiatric Association.

Bar-Haim, Y., Lamy, D., Pergamin, L., Bakermans-Kranenburg, M. J., and van Ijzendoorn, M. H. (2007). Threat-related attentional bias in anxious and non- anxious individuals: a meta-analytic study. *Psychol. Bull.* 133, 1–24. doi: 10.1037/0033-2909.133.1.1

Bastien, C. H., Vallières, A., and Morin, C. M. (2001). Validation of the Insomnia Severity Index as an outcome measure for insomnia research. *Sleep Med.* 2, 297–307. doi: 10.1016/s1389-9457(00)00065-4

Beck, A. T. (1976). *Cognitive Therapy and the Emotional Disorders.* New York, NY: International Universities Press.

Beck, A. T. (1967). *Depression: Clinical, Experimental, and Theoretical Aspects.* New York, NY: Harper & Row.

Bell, C. J., Colhoun, H. C., Frampton, C. M., Douglas, K. M., McIntosh, V. V. W., Carter, F. A., et al. (2017). Earthquake brain: altered recognition and misclassification of facial expressions are related to trauma exposure but not posttraumatic stress disorder. *Front. Psychiatry* 8:278. doi: 10.3389/fpsyt.2017.00278

Bianchini, V., Giusti, L., Salza, A., Cofini, V., Cifone, M. G., Casacchia, M., et al. (2017). Moderate depression promotes posttraumatic growth (Ptg): a young population survey 2 years after the 2009 L'aquila earthquake. *Clin. Pract. Epidemiol. Ment. Health* 13, 10–19. doi: 10.2174/1745017901713010010

Bradley, M. M., and Lang, P. J. (2007). "The international affective picture system (IAPS) in the study of emotion and attention," in *Handbook of Emotion Elicitation and Assessment*, eds J. A. Coan and J. J. B. Allen (Oxford: Oxford University Press), 29–46.

Bradley, M. M., Codispoti, M., Sabatinelli, D., and Lang, P. J. (2001). Emotion and motivation II: sex differences in picture processing. *Emotion* 1, 300–319. doi: 10.1037//1528-3542.1.3.300

Castronovo, V., Galbiati, A., Marelli, S., Brombin, C., Cugnata, F., Giarolli, L., et al. (2016). Validation study of the Italian version of the Insomnia Severity Index (ISI). *Neurol. Sci.* 37, 1517–1524. doi: 10.1007/s10072-016-2620-z

Cheng, Y., Lin, C.-P., Liu, H.-L., Hsu, Y.-Y., Lim, K.-E., Hung, D., et al. (2007). Expertise modulates the perception of pain in others. *Curr. Biol.* 17, 1708–1713. doi: 10.1016/j.cub.2007.09.020

Clarke, P. J., Notebaert, L., and MacLeod, C. (2014). Absence of evidence or evidence of absence: reflecting on therapeutic implementations of attentional bias modification. *BMC Psychiatry* 14:8. doi: 10.1186/1471-244x-14-8

Conson, M., Ponari, M., Monteforte, E., Ricciato, G., Sarà, M., and Trojano, L. (2013). Explicit recognition of emotional facial expressions is shaped by expertise: evidence from professional actors. *Front. Psychol.* 4:382. doi: 10.3389/fpsyg.2013.00382

Dalgleish, T., Taghavi, R., Neshat-Doost, H., Moradi, A., Canterbury, R., and Yule, W. (2003). Patterns of processing bias for emotional information across clinical disorders: a comparison of attention, memory, and prospective cognition in children and adolescents with depression, generalized anxiety, and posttraumatic stress disorder. *J. Clin. Child Adolesc. Psychol.* 32, 10–21. doi: 10.1207/s15374424jccp3201_02

Davis, W. J., Rahman, M. A., Smith, L. J., Burns, A., Senecal, L., McArthur, D., et al. (1995). Properties of the human affect induced by static color slides (IAPS): dimensional, categorical and electromyographic analysis. *Biol. Psychol.* 41, 229–253. doi: 10.1016/0301-0511(95)05141-4

Disner, S. G., Beevers, C. G., Haigh, E. A., and Beck, A. T. (2011). Neural mechanisms of the cognitive model of depression. *Nat. Rev. Neurosci.* 12, 467–477. doi: 10.1038/nrn3027

Ekman, P. (1993). Facial expression and emotion. *Am. Psychol.* 48, 384–392. doi: 10.1037/0003-066X.48.4.384

Ekman, P., and Friesen, W. V. (1976). *Pictures of Facial Affect.* Palo Alto, CA: Consulting Psychologists.

Eysenck, M. W. (1992). *Anxiety: The Cognitive Perspective.* Hove: Psychology Press.

Eysenck, S. B. G., Eysenck, H. J., and Barrett, P. (1985). A revised version of the psychoticism scale. *Pers. Individ. Dif.* 6, 21–29. doi: 10.1016/0191-8869(85)90026-1

Ferrara, M., Mazza, M., Curcio, G., Iaria, G., De Gennaro, L., and Tempesta, D. (2016). Sleep disturbances and spatial memory deficits in post-traumatic stress disorder: the case of L'Aquila (Central Italy). *Epidemiol. Prev.* 40, 45–48. doi: 10.19191/EP16.2S1.P045.047

Freeston, M., Rhéaume, J., Letarte, H., Dugas, M. J., and Ladouceur, R. (1994). Why do people worry? *Pers. Individ. Dif.* 17, 791–802. doi: 10.1016/0191-8869(94)90048-5

Gonda, X., Pompili, M., Serafini, G., Carvalho, A. F., Rihmer, Z., and Dome, P. (2015). The role of cognitive dysfunction in the symptoms and remission from depression. *Ann. Gen. Psychiatry* 14:27. doi: 10.1186/s12991-015-0068-9

Greco, V., and Roger, D. (2001). Coping with uncertainty: the construction and validation of a new measure. *Pers. Individ. Dif.* 31, 519–534. doi: 10.1016/s0191-8869(00)00156-2

Hess, U., and Blairy, S. (2001). Facial mimicry and emotional contagion to dynamic emotional facial expressions and their influence on decoding accuracy. *Int. J. Psychophysiol.* 40, 129–141. doi: 10.1016/S0167-8760(00)00161-6

Labra, O., Maltais, D., and Tremblay, G. (2017). Chilean men exposed to the major earthquake in 2010: investigation of the impacts on their health. *Am. J. Mens Health* 11, 392–403. doi: 10.1177/1557988316681669

Lang, P. J., Bradley, M. M., and Cuthbert, B. N. (1997). *International Affective Picture System (IAPS): Technical Manual and Affective Ratings*. Gainesville, FL: NIMH Center for the Study of Emotion and Attention, University of Florida.

Lang, P. J., Greenwald, M. K., Bradley, M. M., and Hamm, A. O. (1993). Looking at pictures: affective, facial, visceral, and behavioral reactions. *Psychophysiology* 30, 261–273. doi: 10.1111/j.1469-8986.1993.tb03352.x

Lopes, A. P., Macedo, T. F., Coutinho, E. S. F., Figueira, I., and Ventura, P. R. (2014). Systematic review of the efficacy of cognitive-behavior therapy related treatments for victims of natural disasters: a worldwide problem. *PLoS One* 9:e109013. doi: 10.1371/journal.pone.0109013

MacLeod, C., and Clarke, P. J. F. (2013). "Cognitive bias modification: a new frontier in cognition and emotion research," in *Handbook of Cognition and Emotion*, eds M. D. Robinson, E. R. Watkins and E. Harmon-Jones (New York, NY: Guilford), 540–562.

MacLeod, C., and Grafton, B. (2016). Anxiety-linked attentional bias and its modification: illustrating the importance of distinguishing processes and procedures in experimental psychopathology research. *Behav. Res. Ther.* 86, 68–86. doi: 10.1016/j.brat.2016.07.005

MacLeod, C., Mathews, A., and Tata, P. (1986). Attentional bias in emotional disorders. *J. Abnorm. Psychol.* 95, 15–20. doi: 10.1037//0021-843x.95.1.15

Maltais, D., Robichaud, S., and Simard, A. (2001). *Disasters and Disasters*. Chicoutimi: Les éditions JCL.

Mathews, A., and MacLeod, C. (2002). Induced processing biases have causal effects on anxiety. *Cogn. Emot.* 16, 331–354. doi: 10.1080/02699930143000518

Mikels, J. A., Fredrickson, B. L., Larkin, G. R., Lindberg, C. M., Maglio, S. J., and Reuter-Lorenz, P. A. (2005). Emotional category data on images from the International Affective Picture System. *Behav. Res. Methods* 37, 626–630. doi: 10.3758/bf03192732

Neria, Y., Nandi, A., and Galea, S. (2008). Post-traumatic stress disorder following disasters: a systematic review. *Psychol. Med.* 38, 467–480. doi: 10.1017/s0033291707001353

Pedrabissi, L., and Santiniello, M. (1989). *Manuale Dell'Adattamento Italiano Dello STAI Forma Y*. Firenze: Organizzazioni Speciali.

Petrocchi, N., Tenore, K., Couyoumdjian, A., and Gragnani, A. (2015). The anxiety sensitivity index-3: factor structure and psychometric properties in Italian clinical and non-clinical samples. *Boll. Psicol. Appl.* 269, 53–64.

Picconi, L., Jackson, C. J., Balsamo, M., Tommasi, M., and Saggino, A. (2018). Factor structure and measurement invariance across groups of the Italian Eysenck Personality Questionnaire - Short form (EPP-S). *Pers. Individ. Dif.* 123, 76–80. doi: 10.1016/j.paid.2017.11.013

Pistoia, F., Carolei, A., Sacco, S., Conson, M., Pistarini, C., Cazzulani, B., et al. (2015). Contribution of interoceptive information to emotional processing: evidence from individuals with spinal cord injury. *J. Neurotrauma* 32, 1981–1986. doi: 10.1089/neu.2015.3897

Pistoia, F., Conson, M., Trojano, L., Grossi, D., Ponari, M., Colonnese, C., et al. (2010). Impaired conscious recognition of negative facial expressions in patients with locked-in syndrome. *J. Neurosci.* 30, 7838–7844. doi: 10.1523/JNEUROSCI.6300-09.2010

Ponari, M., Trojano, L., Grossi, D., and Conson, M. (2013). "Avoiding or approaching eyes"? Introversion/extraversion affects the gaze-cueing effect. *Cogn. Process.* 14, 293–299. doi: 10.1007/s10339-013-0559-z

Sander, D., Grafman, J., and Zalla, T. (2003). The human amygdala: an evolved system for relevance detection. *Rev. Neurosci.* 14, 303–316. doi: 10.1515/revneuro.2003.14.4.303

Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., and Jacobs, G. A. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.

Tang, B., Deng, Q., Glik, D., Dong, J., and Zhang, L. (2017). A meta-analysis of risk factors for post-traumatic stress disorder (PTSD) in adults and children after earthquakes. *Int. J. Environ. Res. Public Health* 14:E1537. doi: 10.3390/ijerph14121537

Taylor, S., Zvolensky, M. J., Cox, B. J., Deacon, B., Heimberg, R. G., Ledley, D. R., et al. (2007). Robust dimensions of anxiety sensitivity: development and initial validation of the Anxiety Sensitivity Index-3. *Psychol. Assess.* 19, 176–188. doi: 10.1037/1040-3590.19.2.176

Te Brake, H., Dückers, M., De Vries, M., Van Duin, D., Rooze, M., and Spreeuwenberg, C. (2009). Early psychosocial interventions after disasters, terrorism and other shocking events: guideline development. *Nurs. Health Sci.* 11, 336–343. doi: 10.1111/j.1442-2018.2009.00491.x

Tempesta, D., Curcio, G., De Gennaro, L., and Ferrara, M. (2013). Long-term impact of earthquakes on sleep quality. *PLoS One* 8:e55936. doi: 10.1371/journal.pone.0055936

Yule, W. (2001). Posttraumatic stress disorder in the general population and in children. *J. Clin. Psychiatry* 62, 23–28.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership