Published in final edited form as:

Neurobiol Learn Mem. 2018 November ; 155: 42–49. doi:10.1016/j.nlm.2018.05.015.

Contextual fear retrieval-induced Fos expression across early development in the rat: An analysis using established nervous system nomenclature ontology

Anthony J. Santarelli^a, Arshad M. Khan^{b,1}, and Andrew M. Poulos^{a,1,*}

^aDepartment of Psychology, Center for Neuroscience, State University of New York, University at Albany, Albany, NY, 12222, USA

^bUTEP Systems Neuroscience Laboratory, Department of Biological Sciences and Border Biomedical Research Center, University of Texas at El Paso, El Paso, TX, 79968, USA

Abstract

The neural circuits underlying the acquisition, retention and retrieval of contextual fear conditioning have been well characterized in the adult animal. A growing body of work in younger rodents indicates that context-mediated fear expression may vary across development. However, it remains unclear how this expression may be defined across the full range of key developmental ages. Nor is it fully clear whether the structure of the adult context fear network generalizes to earlier ages. In this study, we compared context fear retrieval-induced behavior and neuroanatomically constrained immediate early-gene expression across infant (P19), early and late juvenile (P24 and P35), and adult (P90) male Long-Evans rats. We focused our analysis on neuroanatomically defined subregions and nuclei of the basolateral complex of the amygdala (BLA complex), dorsal and ventral portions of the hippocampus and the subregions of the medial prefrontal cortex as defined by the nomenclature of the Swanson (2004) adult rat brain atlas. Relative to controls and across all ages tested, there were greater numbers of Fos immunoreactive (Fos-ir) neurons in the posterior part of the basolateral amygdalar nuclei (BLAp) following context fear retrieval that correlated statistically with the expression of freezing. However, Fos-ir within regions having known connections with the BLA complex was differentially constrained by developmental age: early juvenile, but not adult rats exhibited an increase of context fear-dependent Fos-ir neurons in prelimbic and infralimbic areas, while adult, but not juvenile rats displayed increases in Fos-ir neurons within the ventral CA1 hippocampus. These results suggest that juvenile and adult rodents may recruit developmentally unique pathways in the acquisition and retrieval of contextual fear. This study extends prior work by providing a broader set of

*Corresponding author at: Department of Psychology, University at Albany, SUNY; Life Sciences Bldg., Room 1058; 1400 Washington Avenue, Albany, NY 12222, USA. apoulos@albany.edu (A.M. Poulos).

¹AMK and AMP are co-senior authors of this study

Disclosure of potential conflicts of interest

The authors have no potential conflicts of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

developmental ages and a rigorously defined neuroanatomical ontology within which the contextual fear network can be studied further.

Keywords

context fear conditioning; development; amygdala; prefrontal cortex; brain atlas; Fos

1. Introduction

A putative neural circuit underlying contextual fear conditioning has been described in young adult animals, 60–120 days of age (Zelikowsky et al., 2014; Orsini et al., 2013; Poulos et al., 2010). In adult rat and mouse, the basolateral complex of the amygdala (BLA complex), dorsal and ventral parts of the hippocampus (dH, vH) and subregions of the medial prefrontal cortex (mPFC) are involved in the acquisition, retention, retrieval and extinction of context mediated fear learning (Fanselow & Poulos, 2005; Kim & Jung, 2006; Rozeske et al., 2015; McCullough et al., 2016). The extent to which neural activity in this network serves the developing organism remains to be fully established, but evidence suggests that context-mediated fear behaviors may recruit and utilize distinct neural pathways during development (Kim & Richardson, 2009; Shechener et al., 2014; Baker et al., 2014; 2016; Callaghan & Richardson, 2014; Jones & Monfils, 2016). Accordingly, we sought to quantify Fos-immunoreactive (Fos-ir) neurons in this context fear neural network in 19-, 24-, 35- and 90-day old male rats using a defined ontology of brain regions to generate novel hypotheses concerning developing context fear neural circuits and to maximize intra- and inter-laboratory reliability of these experimental findings.

In adult mammals, memories of contextual stimuli are established and formed within subfields of the hippocampus (Conejo et al., 2007), which project directly (vH) and indirectly (dH) to nuclei of the BLA complex. Concurrent activation of these and footshock-responsive BLA neurons form the basis of an associative context-fear memory (Schauz & Koch, 2000; Alvarez et al., 2008; Johansen, et al., 2010). Subsequent retrieval of context fear depends upon sufficient reactivation of hippocampal (Raybuck & Lattal, 2014) and BLA neurons, followed by further activation of defensive motor pathways (Amano et al., 2011).

In the developing rat, the expression of context fear emerges around postnatal day (P) 24 (Pugh & Rudy, 1996; Raineke et al., 2010 but see Foster & Burman, 2010; Callaghan & Richardson, 2011). This expression has been largely attributed to the functional emergence of the dH. At these ages as in adult rats, pharmacological inactivation or ablation of the dH has been shown to produce anterograde amnesia for context fear memories, while the protein expression of the immediate early gene (IEG), *c-fos*, within the dH reveals a conditioning dependent increase (Raineke et al., 2010; Foster & Burman, 2010; Schiffino et al., 2011; Robinson-Drummer et al., 2016). However, other reports examining IEG mRNA of *c-Fos* and Early growth response 1 (EGR1) in the dH of juvenile rats have did not identify similar conditioning related effects, which may due to different behavioral procedures employed and/or the ages tested (Asok et al., 2013; Schreiber et al., 2014; Deal et al., 2016; Heroux et al., 2018). Recent work in adult animals has also implicated a compensatory role of medial

prefrontal cortical (mPFC) regions in contextual fear conditioning in animals with pre-training damage to the dH (Zelikowsky et al., 2013). In juvenile rats, pharmacological antagonism of muscarinic and glutamatergic neurotransmitter systems within the mPFC alone can disrupt context fear learning (Heroux et al., 2016; Robinson-Drummer et al., 2017). This function of mPFC may extend to early adulthood (~P60), however its timeline is largely unknown (Chakraborty et al., 2016). Although these observations have been established in juvenile and adult rats independently, the extent to which these observations under conserved conditioning procedures are age-dependent remains to be fully anatomically characterized.

Given the emergence of connectome-based neuroanatomical mapping of neural pathways (Swanson & Lichtman, 2016), a significant and emerging challenge in behavioral neuroscience is the mapping – within a formally defined spatial model of the brain – of molecular expression patterns associated with the functional activation of brain regions during specific behaviors (Watts et al., 2006; also see Bota et al., 2012). Recently, this challenge has been addressed for certain aspects of feeding behavior by Zséli et al., (2016), who mapped expression patterns of Fos, using the formal nervous system nomenclature ontology proposed by Swanson (1992, 1999, 2004, 2018). In the present study, we have begun applying this strategy toward the postnatal development of contextual fear conditioning to examine in infant, juvenile and adult rats the activation of anatomically heterogeneous regions associated with the retrieval of context fear memory. These regions include the hippocampus, mPFC, and BLA complex (including the lateral amygdalar nucleus (LA) and the anterior and posterior portions (BLAa, BLAp) of the basolateral amygdalar nuclei. We hypothesize that delayed but not immediate footshock will result in context fear retrieval in juvenile and adult (Blanchard et al., 1976; Fanselow, 1986; Landeira-Fernandez et al., 2006), but not in infant rats. Furthermore, we hypothesize that this retrieval would correspond to elevations in the numbers of Fos-ir neurons among subregions/nuclei of the hippocampus and basolateral portions (anterior and posterior) of the BLA complex. Finally, we also hypothesize that there is an age-dependent recruitment of the mPFC in the retrieval of context fear, evident by elevated numbers of Fos-ir neurons within juvenile, but not adult animals.

To test these hypotheses, we performed a detailed analysis of contextual fear retrieval and associated neural activation in infant (P19), juvenile (P24 & P35), and adult (P90) rats matched to specific rodent brain atlas plates. Retrieval of context fear was assessed in subjects that received an un-signaled delayed (3 min) or immediate (< 3 sec) footshock. Ninety minutes following context fear testing, subjects were sacrificed, and their brain tissue processed for Fos immunohistochemistry. Nissl-counterstained tissue sections containing the Fos-ir patterns, selected on the basis of their correspondence to specific atlas maps in the Swanson (2004) rat brain atlas, were used to define and parcellate nuclei of the BLA complex, hippocampal formation and prefrontal cortex. As previously described by Zséli et al (2016), we sought to ensure that our semi-quantitative analysis of Fos-IR were performed from comparable tissue representations of each subnuclei, so as to decrease sampling error across subjects (Simmons & Swanson, 2009) of distinct ages. Fos-ir neurons within a single representation of each subnuclei were quantified across training condition and age. Portions of these data have been presented in preliminary form (Santarelli et al., 2016).

2. Materials and methods

Two-day old male Long-Evans rats were cross fostered among dams and randomly assigned to specific testing age groups that were weaned at 21 days of age. For future analysis of neuroanatomical tract tracing not presented in this report, subjects were each anesthetized (3–5% isoflurane) and iontophoretically infused (three days prior to conditioning) with the retrogradely transportable tract tracer, Fluorogold, using a 20- μ m diameter glass micropipette targeting the right BLA complex. Prior to experimental conditioning procedures, subjects on three consecutive days were habituated to handling and transportation procedures. Four groups of animals were used in this experiment: two trained groups and two control groups. The trained groups received a single 1.5 mA footshock, which occurred after a 3-min delay (dS group) or immediately (iS group) upon placement into the conditioning apparatus. The control groups were either exposed to conditioning apparatus, with no footshock (nS group) or remained in the home cage (HMC group). Twenty-four hours later, subjects were returned to the conditioning apparatus for a 4-min test of context fear (dS, iS, nS) or remained in the home cage (HMC). Freezing, defined as the absence of any movement except that related to respiration (Fanselow, 1980), was visually scored by a trained observer blind to the experimental conditions. Ninety minutes after the testing period ended, all subjects were deeply anesthetized and transcardially perfused with 4% *p*-formaldehyde. Brains were extracted, post-fixed in the same fixative for 72 h, and cut into 50 μ m-thick coronal sections. Selected tissue sections were immunostained for Fos protein and counterstained with a fluorescent Nissl stain (see Supplemental Materials for details, including Research Resource Identifiers (RRIDs) for all antibody and counterstaining reagents, per the guidelines of Bandrowski et al., 2016). Brain sections were mounted onto glass slides and scanned at x20 magnification using a fluorescent microscope to examine Nissl and Fos labeling patterns. For each image, the regions and their constituent nuclei were parcellated and analyzed for Fos-ir neurons by trained experimenters blind to the experimental conditions of each subject (for details, see Supplemental Material). The numbers of Fos-ir neurons within a single subnuclei/region representation (50 μ m) for each age group were analyzed by univariate ANOVAs followed by either *post-hoc* (Bonferroni correction) or *planned comparisons* as detailed in Table 1.

3. Results

3.1. Behavioral Analysis

Overall, freezing resulted from a significant interaction between age and condition (Figure 1C) ($F_{(9,87)}=5.042$, $p<0.01$) indicating that contextual fear conditioning increased with developmental age (P19 [dS $n=6$, iS $n=5$, nS $n=7$, HMC $n=4$], P24 [dS $n=5$, iS $n=4$, nS $n=6$, HMC, $n=5$], P35 [dS $n=5$, iS $n=5$, nS $n=6$, HMC $n=7$] and P90 [dS $n=7$, iS $n=6$, nS $n=4$, HMC $n=5$]). In support of our behavioral hypothesis, delayed, but not immediate footshock resulted in a significant increase in context elicited freezing relative to non-shock controls in P24 ($t_{(9)}=1.995$, $p<0.05$), 35 ($t_{(9)}=10.619$, $p<0.01$), and 90 ($t_{(9)}=11.760$, $p<0.01$) rats. Moreover, as predicted, neither dS or iS exhibited significant freezing in P19 rats. In addition, freezing was significantly greater in dS than iS groups in P35 ($t_{(8)}=7.877$, $p<0.01$) and P90 ($t_{(11)}=4.717$, $p<0.01$), but not P24 rats. Post hoc comparisons of dS freezing

between age groups revealed that P90 rats froze to a greater extent than P19 ($p < 0.01$) and P24 rats ($p < 0.05$). This was further supported by a significant linear trend analysis, which found that freezing in the dS, but not iS or nS conditions increased with age ($F_{(3,19)} = 8.940$, $p < 0.01$).

3.2. BLA complex activation

In order to determine context fear retrieval-induced BLA complex neural activation, we assessed Fos-ir within BLAp (Figure 2A), LA, and BLAa (Table 1, Supplemental Figure 2). Separate one-way ANOVAs for the numbers of Fos-ir neurons within the BLAp identified a main effect of condition for each age group [P24 ($F_{(3,20)} = 13.609$, $p < 0.01$); P35 ($F_{(3,19)} = 13.447$, $p < 0.01$); P90 ($F_{(3,23)} = 8.727$, $p < 0.01$)], except P19 rats ($F_{(3,22)} = 0.315$, $p > 0.05$). In support of our hypothesis, P24, P35, and P90 rats – which for the most part all demonstrated context freezing under delayed shock – displayed numbers of Fos-ir neurons within the BLAp that were significantly elevated in dS compared to nS [P24 ($t_{(9)} = 4.453$, $p < 0.01$); P35 ($t_{(9)} = 3.269$, $p < 0.01$); P90 ($t_{(9)} = 3.783$, $p < 0.01$)] conditions. Post-hoc tests revealed significant elevations in the numbers of Fos-ir neurons in dS compared to HMC [P24 ($t_{(10)} = 4.164$, $p < 0.01$); P35 ($t_{(10)} = 10.497$, $p < 0.01$); P90 ($t_{(10)} = 4.1636$, $p < 0.01$) and iS [P24 ($t_{(7)} = 3.448$, $p < 0.05$); P35 ($t_{(8)} = 4.707$, $p < 0.01$)] conditions after correction for multiple comparisons. However, the dS and iS comparison of Fos-ir neuron counts for the P90 rats was not significantly different [P90 ($t_{(10)} = 4.164$, $p < 0.01$)] nS]. In P19 rats, which showed no significant freezing, the numbers of Fos-ir neurons within the BLAp did not differ among any experimental conditions. For each age group, we detected a positive correlation between the numbers of BLAp Fos-ir neurons and freezing (P19 ($r_{(18)} = 0.606$, $p < 0.01$); P24 ($r_{(15)} = 0.636$, $p < 0.05$); P35 ($r_{(16)} = 0.662$, $p < 0.01$); P90 ($r_{(16)} = 0.528$, $p < 0.05$)).

Our hypothesis of context fear retrieval-dependent activation within the BLAa and LA was not supported, as delayed shock did not differ from nS or iS for any of the age groups tested. ANOVAs performed to examine the number of Fos-ir neurons within the BLAa and LA identified a main effect of condition that was limited to P35 rats [BLAa ($F_{(3,19)} = 3.929$, $p < 0.05$); LA ($F_{(3,19)} = 4.564$, $p < 0.05$)]. Post-hoc comparisons revealed that the dS, but not iS nor nS conditions, exhibited a significant increase in the numbers of Fos-ir neurons compared to the HMC condition [BLAa ($t_{(10)} = 3.958$, $p < 0.05$); LA ($t_{(10)} = 5.347$, $p < 0.05$)]. No significant correlations were found between freezing and the numbers of Fos-ir neurons in the LA or BLAa. Importantly, the injection of Fluorogold tracer (see Methods) into the right BLA (results for which are being reported elsewhere: Santarelli et al., 2016), did not produce any obvious impairments in learning, nor was the ability of the ipsilateral BLA complex to mount Fos responses dependent on condition (see Supplemental Figure S1).

3.3. mPFC activation

For the portion of the mPFC we sampled, we counted context fear-retrieval induced Fos-ir neurons in the PL (Figure 2B), ILA (Supplemental Figure 2), and ACA (Table 1). In support of the hypothesis that early juvenile (P24) but not adult rats would show enhanced numbers of PL and ILA Fos-ir neurons that correspond with freezing behavior, we found a main effect of condition within the PL ($F_{(3,20)} = 4.305$, $p < 0.05$) and a trend toward significance for the ILA ($F_{(3,20)} = 3.075$, $p < 0.1$) for P24, but not P90 rats. However, the effects of condition

upon the levels of ACA Fos-ir neurons were not significant for any age groups. Planned comparisons for Fos-ir neurons in the PL revealed that P24 rats showed a significant elevation in the numbers of Fos-ir neurons in dS-trained compared to nS-trained rats within both the PL ($t(9)=3.447$, $p<0.01$) and IL ($t(9)=2.417$, $p<0.05$). Post-hoc comparisons revealed no other significant differences among trained rats within either regions. In P19 rats, analysis for the numbers of Fos-ir neurons within the PL demonstrated a main effect of condition ($F_{(3,212)}=3.205$, $p<0.05$); however, post-hoc tests failed to reveal distinct group differences. P35 rats showed no effects of training condition in either the ILA or PL.

3.4. Hippocampal and extrahippocampal cortical activation

The numbers of Fos-ir neurons that we observed within the dorsal hippocampus (dH) failed to support our hypothesis that context fear retrieval would result in an overall increased neuronal activation in fields CA1. No significant differences were detected across age groups within the dH or its constituent subregions with respect to numbers of dS-specific Fos-ir neurons (Figure 2C, 2D). However, within the vH (Level 37; Figure 2CE), a main effect of condition (elevated numbers of Fos-ir neurons) was observed within field CA1 in P90 ($F_{(3,23)}=3.487$, $p<0.05$), but not P24 or P35 groups. Planned comparisons between dS and nS conditions indicated that the numbers of Fos-ir neurons was elevated in dS animals ($t(9)=3.2646$, $p<0.05$). Unplanned comparisons revealed that dS levels for these neurons were also elevated from HMC ($t(10)=3.935$, $p<0.01$). Interestingly, a main effect of group was also detected for P19 rats ($F_{(3,22)}=3.382$, $p<0.05$). However, post hoc comparisons did not achieve statistical significance.

Within the extra-hippocampal cortical regions we sampled, dS training failed to increase the numbers of Fos-ir neurons relative to those observed in nS and iS groups. P90 rats showed a significant main effect of condition within the PERI ($F_{(3,22)}=5.377$, $p<0.01$) and the rostral portion of the ENTI ($F_{(3,22)}=4.967$, $p<0.05$) (Level 28; Supplemental Figure 2). The numbers of ENTI Fos-ir neurons were elevated in the nS group ($p<0.05$) relative to the HMC group. No other post hoc comparisons of ENTI or PERI achieved statistical significance. A one-way ANOVA analysis for Fos-ir neuron numbers in a more caudal portion of the ENTI did not reveal any main effects at any age.

4. Discussion

The ontogeny of fear retention was examined in this study using a standard contextual fear-conditioning paradigm and was accompanied by a description of induced Fos-ir in brain regions defined within the nervous system nomenclature ontology of Swanson (1992, 1999, 2004, 2017). In agreement with the literature, this study showed that juvenile (P24, P35) and adult rats (P90) are able to express context fear memory following a one-day retention period. Moreover, fear expression involved elevations in the numbers of Fos-ir neurons relative to controls throughout the subregions of the BLA complex that correlated with the observed freezing behavior. In forebrain regions known for their functional links to nuclei of the BLA complex, age-dependent differences were observed in response to conditioning. Specifically, juvenile rats (P24) exposed to delayed-shock displayed an enhancement in the numbers of Fos-ir cells throughout the portions of the PL and ILA that we sampled. In

contrast, adults exhibited such enhancement only within the sampled regions of the ventral CA1 field. Interestingly, P35 rats exhibited a pattern distinct from P24 or P90 suggesting that this age may represent a transition period between the early juvenile and adult rats. Although further analysis of the tissue series used in this study is underway – in which Fos expression is analyzed in neuroanatomically traced circuits among these regions (Santarelli et al., 2016) – these results suggest that the emergence of context fear retrieval in young rats may depend on a functionally activated circuit that is distinct from that activated in the adult.

Many studies have demonstrated a critical role of the hippocampus in context mediated fear behaviors (Fanselow, 2000; Anagnostaras et al., 2001; Matus-Amat et al., 2004; Rudy & Matus-Amat, 2005; Hobin et al., 2006; Rainekei et al., 2010; Jin & Maren, 2015; Xu et al., 2016). In rats, the maturation of the hippocampus continues throughout postnatal life (Bayer, 1980; Gould & Cameron, 1996; Markham et al., 2005). Only recently has evidence appeared for the involvement of the mPFC during the emergence of context fear (Chakraborty et al., 2016; Robinson-Drummer et al., 2017). Indeed, the expression of fear is dependent upon the PL during juvenile development and emerges around P25 (Stella et al., 2012).

While little is known how this circuit changes over development, prefrontal inputs to the BLA subregions are pruned during adolescence (Cressman et al., 2010). However, in adult animals, mPFC involvement in context-fear processing depends on the functional engagement of the dorsal hippocampus: rats with an intact hippocampus do not require mPFC function, while animals with damage to the hippocampus require mPFC–BLA function (Zelikowsky et al., 2013). Adding to this body of work, the present study suggests further that the mPFC involvement is later disengaged in adulthood or may act as a moderator of hippocampal regulation of context fear, as is observed in extinction and renewal (Giustino & Maren, 2015).

A few considerations must be borne in mind with the analysis presented in this study. First, any analysis of Fos-ir *post mortem* is at best a limited proxy of electrical activity of neurons networked *in vivo* (Hoffman & Lyo, 2002; Watts et al., 2006). Notwithstanding this limitation, the data in this study suggest that as in adult animals, the context fear neural system across development is dynamic and can engage anatomically distinct neural pathways (Ponnusamy et al., 2007; Poulos et al., 2009, 2010; Zelikowsky et al., 2013). Second, we report Fos-ir patterns using the nomenclature of the Swanson adult rat brain atlas (2004), in part, because extant atlases of postnatal rat brain (Sherwood & Timiras, 1970; Ramachandra & Subramania, 2011; Calabrese et al., 2013; Khazipov et al., 2015) are restricted primarily to ages earlier than the earliest age we examined here (P19), do not delimit subregional boundaries for certain regions we analyzed, and/or lack the cellular-level spatial resolution required for our analysis. Moreover, a uniform, internally consistent ontology of defined neuroanatomical terms for the rat brain is provided in the nomenclature tables in the Swanson atlases (1992, 1999, 2004, 2018), all of which are based on the same, single rat brain. Using this ontology allows us to contextualize our data in relation to other molecular expression patterns that have been mapped in this spatial model across a variety of behavioral conditions (e.g., Scicli et al., 2004; Yao et al., 2005; Zséli et al., 2016). This approach also ensures that our data can migrate to future atlases of the developing rat that

may utilize the same ontology of defined anatomical terms (see Khan et al., 2018, for a discussion of data migration).

Collectively, the results of the present study along with prior work suggest that the application of a standard adult neural circuit model of context fear conditioning to juvenile (P24 and P35) animals may be an overgeneralization. Our results permit us to propose three key hypotheses: 1) The relative engagement of the vH and mPFC with respect to the BLA is normally regulated by developmental age; 2) in juvenile animals, context-fear processing is biased toward mPFC–BLA pathways and less dependent on vH–BLA pathways; and 3) conversely, in adult animals, context-fear processing is biased toward vH–BLA pathways and less dependent on mPFC–BLA connections.

Support for the first hypothesis is demonstrated by the context fear retrieval dependent activation of the vH in adult, but not early juvenile rats (Figure 2C), while the ILA is activated in juvenile, but not adult rats (Supplemental Figure 2). The second hypothesis is supported by the correlated activation between the PL and ILA with the BLA in juvenile (P24, P35) rats, but not in adult rats. The third hypothesis is supported by research indicating that lesion or inactivation of the adult vH (Rudy & Matus-Amat, 2005; Kim & Cho, 2017), but not the mPFC alone, prevents the adult expression of context fear responding (Zelikowsky et al., 2013). Though another interpretation could be that the relative engagement of these structures is based upon the level of fear acquired or expressed, as P90 rats showed greater freezing than P24 rats. This interpretation seems less likely, when one considers CA1v Fos-ir under similar levels of freezing as in P90 and P35 animals, where the former, but not the latter displayed conditioning related effects. The extent to which the vH and its projections to the BLA are critical in juvenile context-fear conditioning remains to be analyzed. Future work, assessing the neuroanatomical maturation and functional development of vH–BLA and mPFC–BLA pathways in juvenile and adult animals, can test further the role of these pathways in the acquisition, storage and retrieval of context-fear memories.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Kenichiro Negishi, Anais Martinez, and Ellen M. Walker (at UT El Paso) for technical assistance. This work was supported by NIH grants R03MH93781 (AMP) and SC3GM109817 (AMK); and SUNY start-up funds (AMP).

Bibliography

- Alvarez RP, Biggs A, Chen G, Pine DS, Grillon C. 2008; Contextual fear conditioning in humans: cortical-hippocampal and amygdala contributions. *Journal of Neuroscience*. 28(24):6211–6219. DOI: 10.1523/JNEUROSCI.1246-08.2008 [PubMed: 18550763]
- Amano T, Duvarci S, Popa D, Paré D. 2011; The fear circuit revisited: contributions of the basal amygdala nuclei to conditioned fear. *Journal of Neuroscience*. 31(43):15481–15489. DOI: 10.1523/JNEUROSCI.3410-11.2011 [PubMed: 22031894]

- Anagnostaras SG, Gale GD, Fanselow MS. 2001; Hippocampus and contextual fear conditioning: recent controversies and advances. *Hippocampus*. 11(1):8–17. DOI: 10.1002/1098-1063(2001)11:1<8::AID-HIPO1015>3.0.CO;2-7 [PubMed: 11261775]
- Asok A, Schreiber WB, Jablonski SA, Rosen JB, Stanton ME. 2013; Egr-1 increases in the prefrontal cortex following training in the context pre-exposure facilitation effect (CPFE) paradigm. *Neurobiology of Learning and Memory*. 106:145–153. DOI: 10.1016/j.nlm.2013.08.006 [PubMed: 23973447]
- Baker KD, Bisby MA, Richardson R. 2016; Impaired fear extinction in adolescent rodents: Behavioural and neural analyses. *Neuroscience and Biobehavioral Reviews*. 70:59–73. DOI: 10.1016/j.neubiorev.2016.05.019 [PubMed: 27235077]
- Baker KD, Den ML, Graham BM, Richardson R. 2014; A window of vulnerability: impaired fear extinction in adolescence. *Neurobiology of Learning and Memory*. 113:90–100. DOI: 10.1016/j.nlm.2013.10.009 [PubMed: 24513634]
- Bandrowski A, Brush M, Grethe JS, Haendel MA, Kennedy DN, Hill S, Hof PR, Martone ME, Pols M, Tan SS, Washington N, Zudilova-Seinstra E, Vasilevsky N. RINL Resource Identification Initiative. 2016; The Resource Identification Initiative: A cultural shift in publishing. *Neuroinformatics*. 14(2):169–182. DOI: 10.1007/s12021-015-9284-3 [PubMed: 26589523]
- Bayer SA. 1980; Development of the hippocampal region in the rat. II. Morphogenesis during embryonic and early postnatal life. *Journal of Comparative Neurology*. 190:115–134. DOI: 10.1002/cne.901900108 [PubMed: 7381049]
- Beck CH, Fibiger HC. 1995; Conditioned fear induced changes in behavior and in the expression of the immediate early gene c-fos: with and without diazepam pretreatment. *The Journal of Neuroscience*. 15(1):709–20. [PubMed: 7823174]
- Blackstad TW. 1956; Commissural connections of the hippocampal region in the rat, with special reference to their mode of termination. *Journal of Comparative Neurology*. 105(3):417–537. DOI: 10.1002/cne.901050305 [PubMed: 13385382]
- Blanchard RJ, Fukunaga KK, Blanchard DC. 1976; Environmental control of defensive reactions to footshock. *Bulletin of the Psychonomic Society*. 8:129–130.
- Bota M, Sporns O, Swanson LW. 2012; Neuroinformatics analysis of molecular expression patterns and neuron populations in gray matter regions: The rat BST as a rich exemplar. *Brain Research*. 1450:174–193. DOI: 10.1016/j.brainres.2012.02.034 [PubMed: 22421015]
- Calabrese E, Badea A, Watson C, Johnson GA. 2013; A quantitative magnetic resonance histology atlas of postnatal rat brain development with regional estimates of growth and variability. *Neuroimage*. 71:196–206. DOI: 10.1016/j.neuroimage.2013.01.017 [PubMed: 23353030]
- Callaghan BL, Richardson R. 2014; Early emergence of adult-like fear renewal in the developing rat after chronic corticosterone treatment of the dam or the pups. *Behavioral Neuroscience*. 128(5):594–602. DOI: 10.1037/bne0000009 [PubMed: 25150542]
- Chakraborty T, Asok A, Stanton ME, Rosen JB. 2016; Variants of contextual fear conditioning induce differential patterns of Egr-1 activity within the young adult prefrontal cortex. *Behavioral Brain Research*. 302:122–130. DOI: 10.1016/j.bbr.2016.01.018
- Conejo NM, Gonzalez-Pardo H, Lopez M, Cantora R, Arias JL. 2007; Induction of c-Fos expression in the mammillary bodies, anterior thalamus and dorsal hippocampus after fear conditioning. *Brain Research Bulletin*. 74(1–3):172–177. DOI: 10.1016/j.brainresbull.2007.06.006 [PubMed: 17683804]
- Cressman VL, Balaban J, Steinfeld S, Shemyakin A, Graham P, Parisot N, Moore H. 2010; Prefrontal cortical inputs to the basal amygdala undergo pruning during late adolescence in the rat. *Journal of Comparative Neurology*. 518(14):2693–709. DOI: 10.1002/cne.22359 [PubMed: 20506471]
- Deal AL, Erickson KJ, Shiers SI, Burman MA. 2016; Limbic system development underlies the emergence of classical fear conditioning during the 3rd and 4th weeks of life in the rat. *Behav Neurosci*. 130(2):212–30. [PubMed: 26820587]
- Fanselow MS. 1980; Conditioned and unconditional components of post-shock freezing. *Pavlovian Journal of Biological Sciences*. 15(4):177–182.

- Fanselow MS. 1986; Associative vs topographical accounts of the immediate shock freezing deficit in rats: Implication for the response selection rules governing species specific defensive reactions. *Learning and Motivation*. 17:16–39.
- Fanselow MS. 2000; Contextual fear, gestalt memories, and the hippocampus. *Behavioral Brain Research*. 110(1–2):73–81. DOI: 10.1016/S0166-4328(99)00186-2
- Fanselow MS, Poulos AM. 2005; The neuroscience of mammalian associative learning. *Annual Review of Psychology*. 56:207–234. DOI: 10.1146/annurev.psych.56.091103.070213
- Foster JA, Burman MA. 2010; Evidence for hippocampus-dependent contextual learning at postnatal day 17 in the rat. *Learning and Memory*. 17(5):259–266. DOI: 10.1101/lm.1755810 [PubMed: 20427514]
- Giustino TF, Maren S. 2015; The role of the medial prefrontal cortex in the conditioning and extinction of fear. *Frontiers in Behavioral Neuroscience*. 9:298.doi: 10.3389/fnbeh.2015.00298 [PubMed: 26617500]
- Gould E, Cameron HA. 1996; Regulation of neuronal birth, migration and death in the rat dentate gyrus. *Developmental Neuroscience*. 18(1–2):22–35. [PubMed: 8840084]
- Heroux NA, Osborne BF, Miller LA, Kawan M, Buban KN, Rosen JB, Stanton ME. 2018; Differential expression of the immediate early genes *c-Fos*, *Arc*, *Egr-1*, and *Npas4* during long-term memory formation in the context preexposure facilitation effect (CPFE). *Neurobiology of Learning and Memory*. 147:128–138. DOI: 10.1016/j.nlm.2017.11.016 [PubMed: 29222058]
- Hobin JA, Ji J, Maren S. 2006; Ventral hippocampal muscimol disrupts context-specific fear memory retrieval after extinction in rats. *Hippocampus*. 16(2):174–182. DOI: 10.1002/hipo.20144 [PubMed: 16358312]
- Hoffman GE, Lyo D. 2002; Anatomical markers of activity in neuroendocrine systems: Are we all ‘Fos-ed out’? *Journal of Neuroendocrinology*. 14:259–268. DOI: 10.1046/j.1365-2826.2002.00775.x [PubMed: 11963822]
- Jin J, Maren S. 2015; Fear renewal preferentially activates ventral hippocampal neurons projecting to both amygdala and prefrontal cortex in rats. *Scientific Reports*. 5:8388.doi: 10.1038/srep08388 [PubMed: 25669753]
- Jones CE, Monfils MH. 2016; Post-retrieval extinction in adolescence prevents return of juvenile fear. *Learning and Memory*. 23(10):567–575. DOI: 10.1101/lm.043281.116 [PubMed: 27634147]
- Johansen JP, Tarpley JW, LeDoux JE, Blair HT. 2010; Neural substrates for expectation-modulated fear learning in the amygdala and periaqueductal gray. *Nature Neuroscience*. 13(8):979–986. DOI: 10.1038/nn.2594 [PubMed: 20601946]
- Khan AM, Perez J, Wells CE, Fuentes O. 2018; Computer vision evidence supporting craniometric alignment of rat brain atlases to streamline expert-guided, first-order migration of hypothalamic spatial datasets related to behavioral control. *Frontiers in Systems Neuroscience*. doi: 10.3389/fnsys.2018.00007
- Khazipov R, Zaynutdinova D, Ogievetsky E, Valeeva G, Mitrukina O, Manent JB, Represa A. 2015; Atlas of the postnatal rat brain in stereotaxic coordinates. *Frontiers in Neuroanatomy*. 9:161.doi: 10.3389/fnana.2015.00161 [PubMed: 26778970]
- Kim JH, Richardson R. 2009; Fear extinction across development: the involvement of the medial prefrontal cortex as assessed by temporary inactivation and immunohistochemistry. *Journal of Neuroscience*. 29(35):10802–10808. DOI: 10.1523/JNEUROSCI.0596-09.2009 [PubMed: 19726637]
- Kim JJ, Jung MW. 2006; Neural circuits and mechanisms involved in Pavlovian fear conditioning: a critical review. *Neuroscience and Biobehavioral Reviews*. 30(2):188–202. DOI: 10.1016/j.neubiorev.2005.06.005 [PubMed: 16120461]
- Kim WB, Cho JH. 2017; Synaptic targeting of double-projecting ventral CA1 hippocampal neurons to the medial prefrontal cortex and basal amygdala. *Journal of Neuroscience*. 37(19):4868–4882. DOI: 10.1523/JNEUROSCI.3579-16.2017 [PubMed: 28385873]
- Krieg WJ. 1946; Connections of the cerebral cortex; the albino rat; topography of the cortical areas. *Journal of Comparative Neurology*. 84:221–275. [PubMed: 20982805]

- Krettek JE, Price JL. 1977; Projections from the amygdaloid complex to the cerebral cortex and thalamus in the rat and cat. *Journal of Comparative Neurology*. 172(4):687–722. DOI: 10.1002/cne.901720408 [PubMed: 838895]
- Krettek JE, Price JL. 1978; A description of the amygdaloid complex in the rat and cat with observation on intra-amygdaloid axonal connections. *Journal of Comparative Neurology*. 178(2): 255–280. DOI: 10.1002/cne.901780205 [PubMed: 627626]
- Landeira-Fernandez J, DeCola JP, Kim JJ, Fanselow MS. 2006; Immediate shock deficit in fear conditioning: effects of shock manipulations. *Behavioral Neuroscience*. 120(4):873–879. [PubMed: 16893293]
- Markham JA, McKian KP, Stroup TS, Juraska JM. 2005; Sexually dimorphic aging of dendritic morphology in CA1 of hippocampus. *Hippocampus*. 15(1):97–103. DOI: 10.1002/hipo.20034 [PubMed: 15390161]
- Matus-Amat P, Higgins EA, Barrientos RM, Rudy JW. 2004; The role of the dorsal hippocampus in the acquisition and retrieval of context memory representations. *Journal of Neuroscience*. 24(10): 2431–2439. DOI: 10.1523/JNEUROSCI.1598-03.2004 [PubMed: 15014118]
- McCullough KM, Morrison FG, Ressler KJ. 2016; Bridging the gap: Towards a cell-type specific understanding of neural circuits underlying fear behaviors. *Neurobiology of Learning and Memory*. 135:27–39. DOI: 10.1016/j.nlm.2016.07.02 [PubMed: 27470092]
- Orsini CA, Yan C, Maren S. 2013; Ensemble coding of context-dependent fear memory in the amygdala. *Frontiers in Behavioral Neuroscience*. 7:199.doi: 10.3389/fnbeh.2013.00199.eCollection2013 [PubMed: 24379767]
- Ponnusamy R, Poulos AM, Fanselow MS. 2007; Amygdala-dependent and amygdala-independent pathways for contextual fear conditioning. *Neuroscience*. 147(4):919–927. DOI: 10.1016/j.neuroscience.2007.04.026 [PubMed: 17587502]
- Poulos AM, Li V, Sterlace SS, Tokushige F, Ponnusamy R, Fanselow MS. 2009; Persistence of fear memory across time requires the basolateral amygdala complex. *Proceedings of the National Academy of Sciences, USA*. 160(28):11737–11741. DOI: 10.1073/pnas.0905257106
- Poulos AM, Ponnusamy R, Dong HW, Fanselow MS. 2010; Compensation in the neural circuitry of fear conditioning awakens learning circuits in the bed nuclei of the stria terminalis. *Proceedings of the National Academy of Sciences, USA*. 107(33):14881–14886. DOI: 10.1073/pnas.1005754107
- Pugh CR, Rudy JW. 1996; A developmental analysis of contextual fear conditioning. *Developmental Psychobiology*. 29(2):87–100. DOI: 10.1002/(SICI)1098-2302(199603)29:2<87::AID-DEV1>3.0.CO;2-H [PubMed: 8919089]
- Raineki C, Holman PJ, Debiec J, Bugg M, Beasley A, Sullivan RM. 2010; Functional emergence of the hippocampus in context fear learning in infant rats. *Hippocampus*. 20(9):1037–1046. DOI: 10.1002/hipo.20702 [PubMed: 19739248]
- Ramachandra, R, Subramanian, T. *Atlas of the neonatal rat brain*. Boca Raton, FL: CRC Press; 2011.
- Raybuck JD, Lattal KM. 2014; Differential effects of dorsal hippocampal inactivation on expression of recent and remote drug and fear memory. *Neuroscience Letters*. 569:1–5. DOI: 10.1016/j.neulet.2014.02.063 [PubMed: 24686177]
- Robinson-Drummer PA, Dokovna LB, Heroux NA, Stanton ME. 2016; Cholinergic mechanisms of the context preexposure facilitation effect in adolescent rats. *Behavioral Neuroscience*. 130(2):196–205. DOI: 10.1037/bne0000134 [PubMed: 26866360]
- Robinson-Drummer PA, Heroux NA, Stanton ME. 2017; Antagonism of muscarinic acetylcholine receptors in medial prefrontal cortex disrupts the context preexposure facilitation effect. *Neurobiology of Learning and Memory*. 143:27–35. DOI: 10.1016/j.nlm.2017.04.003 [PubMed: 28411153]
- Rozeske RR, Valerio S, Chaudun F, Herry C. 2015; Prefrontal neuronal circuits of contextual fear conditioning. *Genes, Brain and Behavior*. 14(1):298–306. DOI: 10.1111/gbb.12181
- Rudy JW, Matus-Amat P. 2005; The ventral hippocampus supports a memory representation of context and contextual fear conditioning: implications for a unitary function of the hippocampus. *Behavioral Neuroscience*. 119(1):154–163. DOI: 10.1037/0735-7044.119.1.154 [PubMed: 15727521]

- Santarelli, AJ; Negishi, KN; Khan, AM; Poulos, AM. Neural tract tracing-based modeling of contextual fear circuits across development in rats. Program No. 452.15. 2016 Neuroscience Meeting Planner; San Diego, CA. 2016; Society for Neuroscience; 2016. Online
- Schauz C, Koch M. 2000; Blockade of NMDA receptors in the amygdala prevents latent inhibition of fear-conditioning. *Learning and Memory*. 7(6):393–399. DOI: 10.1101/lm.33800 [PubMed: 11112798]
- Schiffino FL, Murawski NJ, Rosen JB, Stanton ME. 2011; Ontogeny and Neural substrates of the context pre-exposure facilitation effect. *Neurobiology of Learning and Memory*. 95(2):190–198. DOI: 10.1016/j.nlm.2010.11.011 [PubMed: 21129493]
- Schreiber WA, Asok A, Jablonski SA, Rosen JB, Stanton ME. 2014; Egr-1 mRNA expression patterns in the prefrontal cortex, hippocampus, and amygdala during variants of contextual fear conditioning in adolescent rats. *Brain Research*. 12(1576):63–72. DOI: 10.1016/j.brainres.2014.06.007
- Scicli AP, Petrovich GD, Swanson LW, Thompson RF. 2004; Contextual fear conditioning is associated with lateralized expression of the immediate early gene c-fos in the central and basolateral amygdalar nuclei. *Behavioral Neuroscience*. 118(1):5–14. [PubMed: 14979778]
- Shechner T, Hong M, Britton JC, Pine DS, Fox NA. 2014; Fear conditioning and extinction across development: evidence from human studies and animal models. *Biological Psychology*. 100:1–12. DOI: 10.1016/j.biopsycho.2014.04.001 [PubMed: 24746848]
- Sherwood, N, Timiras, P. A stereotaxic atlas of the developing rat brain. Berkeley: University of California Press; 1970.
- Stella F, Cerasti E, Si B, Jazek K, Treves A. 2012; Self-organization of multiple spatial and context memories in the hippocampus. *Neuroscience and Biobehavioral Reviews*. 36(7):1609–1625. DOI: 10.1016/j.neubiorev.2011.12.002 [PubMed: 22192880]
- Swanson, LW, Kohler, C, Bjorklund, A. The limbic region. I: The septohippocampal system. In: Bjorklund, A, Hökfelt, T, Swanson, LW, editors *Handbook of Chemical Neuroanatomy*, Vol. 5 Integrated Systems of the CNS, Part I. Elsevier; New York: 1987. 125–277.
- Swanson, LW. *Brain Maps: Structure of the rat brain*. Amsterdam: Elsevier; 1992.
- Swanson, LW. *Brain Maps: Structure of the rat brain*. 2. Amsterdam: Elsevier; 1999.
- Swanson LW. 2003; The amygdala and its place in the cerebral hemisphere. *Annals of the New York Academy of Sciences*. 985:174–184. DOI: 10.1111/j.1749-6632.2003.tb07081.x [PubMed: 12724158]
- Swanson, LW. *Brain Maps: Structure of the rat brain*. 3. Amsterdam: Elsevier; 2004.
- Swanson LW. 2018; *Brain Maps 4.0 Structure of the rat brain*: An open access atlas with global nervous system nomenclature ontology and flatmaps. *Journal of Comparative Neurology*. 526(6): 935–943. DOI: 10.1002/cne.24381 [PubMed: 29277900]
- Swanson LW, Lichtman JW. 2016; From Cajal to connectome and beyond. *Annual Reviews of Neuroscience*. 39(1):197–216. DOI: 10.1146/annurev-neuro-071714-033954
- Swanson LW, Petrovich GD. 1998; What is the amygdala? *Trends in Neurosciences*. 21(8):323–331. DOI: 10.1016/S0166-2236(98)01265-X [PubMed: 9720596]
- Vogt BA, Peters A. 1981; Form and distribution of neurons in the rat cingulate cortex: areas 32, 24, and 29. *Journal of Comparative Neurology*. 195(4):603–625. DOI: 10.1002/cne.901950406 [PubMed: 7462444]
- Watts AG, Khan AM, Sanchez-Watts G, Salter D, Neuner CM. 2006; Activation in neural networks controlling ingestive behaviors: What does it mean, and how do we map and measure it? *Physiology and Behavior*. 89:501–510. DOI: 10.1016/j.physbeh.2006.05.025 [PubMed: 16828817]
- Xu C, Krabbe S, Grundemann J, Botta P, Fadok JP, Osakada F, et al. 2016; Distinct hippocampal pathways mediate dissociable roles of context in memory retrieval. *Cell*. 167(4):961–972. DOI: 10.1016/j.cell.2016.09.051 [PubMed: 27773481]
- Yao ST, Gouraud S, Paton JFR, Murphy D. 2005; Water deprivation increases the expression of neuronal nitric oxide synthase (nNOS) but not orexin-A in the lateral hypothalamic area of the rat. *Journal of Comparative Neurology*. 490(2):180–193. DOI: 10.1002/cne.20662 [PubMed: 16052497]

- Zelikowsky M, Bissiere S, Hast TA, Bennett RZ, Abdipranoto A, Vissel B, Fanselow MS. 2013; Prefrontal microcircuit underlies contextual learning after hippocampal loss. *Proceedings of the National Academy of Sciences, USA*. 110(24):9938–9843. DOI: 10.1073/pnas.1301691110
- Zelikowsky M, Hersman S, Chawla MK, Barnes CA, Fanselow MS. 2014; Neuronal ensembles in amygdala, hippocampus, and prefrontal cortex track differential components of contextual fear. *Journal of Neuroscience*. 34(25):8462–8466. DOI: 10.1523/JNEUROSCI.3624-13.2014 [PubMed: 24948801]
- Zséli G, Vida B, Martinez A, Lechan RM, Khan AM, Fekete C. 2016; Elucidation of the anatomy of a satiety network: Focus on connectivity of the parabrachial nucleus in the adult rat. *Journal of Comparative Neurology*. 524(14):2803–2827. DOI: 10.1002/cne.23992 [PubMed: 26918800]

Figure 1.

Study design and behavioral testing results: A: Subjects were randomly assigned into either the HMC (home cage), nS (no shock), iS (immediate-shock), or dS (delayed-shock) groups. Context fear retrieval was assessed 24 h post-conditioning and was followed by sacrifice and perfusion of the experimental animals. B: Representative examples of the sections mapped to corresponding Swanson (2004) atlas levels 8, 28 and 38. C: Context fear retrieval represented as a total mean percentage of time spent freezing (+ SEM) during a 4-min context fear test. Asterisks (*) denote significance at the $p < 0.05$ threshold. The number sign (#) denotes a trend towards significance at the $p < 0.1$ threshold. The open access maps in Panel B are reproduced from Swanson, L.W. (2004) *Brain maps: structure of the rat brain*, 3rd edition; under the terms of a Creative Commons Attribution-NonCommercial 4.0 International License (<https://creativecommons.org/licenses/by-nc/4.0/>) and are available for download at larryswanson.com.

Figure 2.

The average numbers of context fear retrieval induced Fos-ir neurons + SEM as a percent of average numbers of Fos-ir neurons in the home cage condition (Fos-ir, %HMC) quantified at 1 d following initial conditioning by a 1.5 mA footshock. A–C: Fos-ir %HMC quantified in sampled subregions within the BLAp (A), PL (B) and CA1v (C). D–F: Representative photomicrographs of coronal sections for Fos-ir (green) and the background Nissl stain, Neuro-Trace (magenta), depicting BLAp (D), PL (E) and CA1v (F) among dS and nS conditions. Asterisks (*) denote significance at the $p < 0.05$ threshold for *a priori* tests of the hypothesis. The number sign (#) denotes a trend towards significance at the $p < 0.1$ threshold for *a priori* tests of the hypothesis. Scale bars in D–F 50 μ M.

Statistical analysis: structure and results. Illustrated is the sequence of analysis with *a-priori* tests followed by exploratory one-way ANOVAs. Bonferroni corrected multiple comparisons were completed following significant ANOVAs.

Table 1

Planned Comparisons dS vs. nS					
P24			P35		
Region	T-Value	Significance	T-Value	Significance	Significance
<i>BLAa</i>	1.2584	ns	1.1154	ns	ns
<i>BLAp</i>	4.453	p<0.01	3.269	p<0.01	p<0.01
<i>CAIv</i>	0.9198	ns	0.2687	ns	p<0.05
<i>CAId</i>	0.4219	ns	0.0062	ns	ns
<i>PL</i>	3.447	p<0.01			
<i>IL</i>	2.417	p<0.05			
Exploratory Univariate ANOVA Comparisons					
P19			P24		
Region	Univariate F	Significance	Univariate F	Significance	Significance
<i>BLAa</i>	F(3,22)=0.199	ns	F(3,20)=2.191	ns	F(3,23)=4.518
<i>BLAp</i>	F(3,22)=0.315	ns	F(3,20)=13.609	p<0.01	F(3,23)=13.447
<i>CAIv</i>	F(3,22)=3.382	p<0.05	F(3,20)=1.019	ns	F(3,23)=2.872
<i>CAId</i>	F(3,22)=0.202	ns	F(3,20)=1.057	ns	F(3,23)=0.697
<i>PL</i>	F(3,22)=3.205	p<0.05	F(3,20)=4.305	p<0.05	F(3,23)=0.702
<i>IL</i>	F(3,22)=1.096	ns	F(3,20)=3.075	p<0.1	F(3,23)=0.240
<i>ACA</i>	F(3,22)=1.06	ns	F(3,20)=0.567	ns	F(3,23)=1.263
<i>LA</i>	F(3,22)=0.051	ns	F(3,20)=2.451	ns	F(3,23)=4.564
<i>dCA3</i>	F(3,22)=0.572	ns	F(3,20)=0.579	ns	F(3,23)=0.441
<i>dDG</i>	F(3,22)=2.493	ns	F(3,20)=0.335	ns	F(3,23)=0.733
<i>nENTI</i>	F(3,22)=0.464	ns	F(3,20)=0.982	ns	F(3,23)=0.844
<i>PERI</i>	F(3,22)=0.831	ns	F(3,20)=1.609	ns	F(3,23)=2.303
<i>vCA3</i>	F(3,22)=3.116	p<0.1	F(3,20)=0.648	ns	F(3,23)=0.627
					F(3,22)=2.952
					F(3,22)=8.727
					F(3,22)=3.415
					F(3,22)=0.920
					F(3,22)=0.747
					F(3,22)=0.379
					F(3,22)=0.485
					F(3,22)=2.662
					F(3,22)=0.114
					F(3,22)=1.579
					F(3,22)=4.967
					F(3,22)=5.377
					F(3,22)=1.05

Planned Comparisons dS vs. nS									
P24			P35			P90			
Region	T-Value	Significance	T-Value	Significance		T-Value	Significance		
cENTI	F(3,22)=0.564	ns	F(3,20)=0.839	ns	F(3,23)=3.031	p<0.1	F(3,22)=3.049	p<0.1	
Bonferroni Corrected Multiple Comparisons									
dS vs. HMC									
P19 CAIv	ns		ns	ns	dS vs. iS	iS vs. HMC	iS vs. nS	ns vs. HMC	
PL	ns		ns	ns					ns
P24 BLAp	p<0.01			p<0.01					ns
PL	ns			ns					ns
P35 BLAa	P<0.05			ns					ns
BLAp	p<0.01			p<0.05					ns
LA	p<0.05		ns	ns					ns
cENTI	ns		ns	ns					ns
P90 BLAp	p<0.01			ns		p<0.05			ns
CAIv	p<0.05			ns		ns			ns
rENTI	p<0.05		ns	ns		p<0.05			p<0.05
PERI	p<0.01		ns	ns		ns			p<0.05
Correlations									
BLAa-Freezing									
P19	r=0.153		r=0.606	r=0.424	r=0.060	r=-0.041	r=0.093		
	ns		p<0.01	ns	ns	ns	ns		
P24	r=0.031		r=0.636	r=0.041	r=0.500	r=0.272	r=-0.273		
	ns		p<0.05	ns	p<0.1	ns	ns		
P35	r=0.179		r=0.662	r=0.341	r=0.504	r=0.490	r=0.222		
	ns		p<0.01	ns	p<0.05	p<0.1	ns		

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Planned Comparisons dS vs. nS					
	P90		P35		P24
Region	T-Value	Significance	T-Value	Significance	T-Value
P90					
	r=0.154	ns	r=-0.024	ns	r=0.012
	r=0.358	ns	r=-0.528	p<0.05	ns
	r=0.459	p<0.1			