HHS Public Access

Author manuscript

Brain Res. Author manuscript; available in PMC 2019 January 01.

Published in final edited form as:

Brain Res. 2018 January 01; 1678: 214–219. doi:10.1016/j.brainres.2017.10.028.

Hemispheric differences in the number of parvalbumin-positive neurons in subdivisions of the rat basolateral amygdala complex

Ryan K. Butler[1,*], **Elisabeth M. Oliver**, **Jim R. Fadel**, and **Marlene A. Wilson**

Department of Pharmacology, Physiology and Neuroscience, University of South Carolina School of Medicine, Columbia, SC, USAWJB Dorn Veterans Affairs Medical Center, Columbia SC

Abstract

The amygdala is a bilateral temporal lobe brain region which plays an important role in emotional processing. Past studies on the amygdala have shown hemispheric differences in amygdalar processes and responses associated with specific pain and fear behaviors. Despite the functional differences in the amygdala, few studies have been performed to characterize whether anatomical differences exist between the left and right amygdala. Parvalbumin (PV) is a phenotypic marker for an inhibitory interneuronal population in cortical brain structures such as the basolateral amygdala complex (BLC). This study examined the number of PV-positive neurons in the left and right BLC of adult, male Long-Evans rats using unbiased stereology. Coronal sections through the rostral-caudal extent of the BLC were immunohistochemically-stained for PV and the optical fractionator method was used to obtain an unbiased estimate of the number of PV-positive neurons in subdivisions through the BLC. The lateral and basolateral amygdala divisions of the BLC were analyzed, were subdivided into the dorsolateral, ventrolateral and ventromedial and the posterior, anterior and ventral subdivisions, respectively. The results indicate that there are significantly more PV-positive neurons in the left basolateral amygdala compared to the right, with a significant difference specifically in the posterior subdivision. This difference in PV neuronal number could help explain the distinct hemispheric roles of the BLC in the behavioral processing following exposure to painful and fearful stimuli.

1. Introduction

The amygdala is a bilateral temporal lobe brain region which plays an important role in emotional processing. Past studies on the amygdala have shown hemispheric differences in amygdalar processes and responses associated with specific pain and fear behaviors. The basolateral amygdala (in this study known as the basolateral amygdala complex-BLC) includes the lateral, basolateral and basomedial nuclei (Augustine, 2017; Patestas and Gartner, 2016; Sah et al., 2003). The BLC is a cortical-like structure that can also be divided

[1]Correspondence: Dr. Ryan K. Butler, Bowles Center for Alcohol Studies, Thurston Bowles Building, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7178, USA, USA, Tel: + 1 919 966 3081, ryan.butler@med.unc.edu.

[*]Current Address: Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

into multiple subdivisions, including the dorsolateral, ventrolateral and ventromedial subdivisions of the lateral amygdala, and the posterior, anterior and ventral divisions of the basolateral amygdala (Sah et al., 2003). Despite the hemispheric asymmetry in functionality between the left and right BLC, a paucity of data exists which have examined anatomical differences between the two hemispheres of the BLC.

In humans, activation in the BLC and other parts of the amygdala is lateralized during painful or fearful stimuli; however, different reports have demonstrated that the left or right BLC is preferentially activated based on the type of painful or fearful stimuli (Ji and Neugebauer, 2009). A Baas *et al.* (2004) review on human imaging studies and found that the the right amygdala was activated more in emotional memory creation (an unconscious event), but left amygdala was activated primarily in emotional processing (a conscious event). Studies have also been done on humans with unilateral amygdalar lesions (Adolphs et al., 2000; Buchanan et al., 2001). These studies specifically investigated emotional memory formation in subjects with unilateral lesions versus controls. In both studies subjects with lesions in the left amygdala had significantly less recall of the arousing stimulus (words or pictures) than both the right amygdala lesion and control groups. These data support the theory that left amygdala is more important in cognitive and intentional emotional processing than the right amygdala. In addition, individuals with social anxiety disorder had more amygdalar activation in the right amygdala when observing a neutral face compared to control subjects that showed had more activation in the left amygdala (Cooney et al., 2006), supporting the notion of hemispheric asymmetry between conscious and unconscious emotional processing.

Lateralization has been shown in the rodent and cat BLC in different aspects of unconditioned and conditioned fear responses. This lateralization has been demonstrated to be receptor-specific and mediated by multiple signal transduction pathways. Predator stress leads to long-term changes in multiple responses including acoustic startle and anxiety-like behaviors in the elevated plus maze, and studies from the Adamec group suggest that there is lateralized control of these glutamatergic processes over long-term stress effects. N-methyl-D-aspartate (NMDA) antagonists in the right amygdala had greater effects on increases in acoustic startle responses induced by predator stress than injections in left amygdala. In contrast, NMDA receptor blockade in the left BLC blocked predator-induced decreases in risk assessment, but not open arm time, in the elevated plus maze (Adamec et al., 1999b). This intriguing study suggests that glutamatergic processes associated with stress in amygdala may exert very specific influences on distinct behavioral endpoints, even during the same task, and that there is a lateralized control of these glutamatergic processes over stress effects (for review see Wilson et al., 2015). Adamec et al. (2004) also found that the baseline anxiety levels of rats before kindling in the right BLC determined whether the rats would be more or less anxious after kindling – an effect not shown in the left BLC. Further, the partial kindling response of cats is dependent on long-term potentiation of the right amygdala, but not the left (Adamec, 1999). Neurochemical changes in conditioning paradigms also show lateralized effects. For example, lower levels of protein kinase C beta II (PKCβII) are seen in the right BLC compared to the left BLC of rats when presented with paired tone and footshock, or the unconditioned controls (no tone or footshock) (Orman and Stewart, 2007). Interestingly, PKCβII levels were increased in the right BLC of rats

(compared to the left BLC) receiving randomly presented tone and shocks. Altogether, the data on lateralization of function of the BLC in rodents has demonstrated remarkable specification of discrete aspects of unconditioned and conditioned fear behaviors and that these effects are mediated by distinct receptor systems and signal transduction pathways.

Typical of cortical brain regions, the calcium-binding protein parvalbumin (PV) is present in a subset of the inhibitory, interneuronal populations of the BLC (Celio, 1986; Celio, 1990; Kemppainen and Pitkanen, 2000; Mascagni and McDonald, 2009; McDonald and Mascagni, 2002; McDonald et al., 2012; Sorvari et al., 1995). Furthermore, PV-positive neurons in the BLC project directly to pyramidal, glutamatergic neurons of the BLC which contain calcium/calmodulin-dependent protein kinase (CAMK) II (Muller et al., 2006). PV-positive neurons in the right BLC displayed heterogeneous firing in response to a noxious stimuli, similar to the pattern displayed by CAMKII-positive neurons of the right BLC (Bienvenu et al., 2012). It is possible that the asymmetric functionality of the BLC can be attributed to a variance in numbers of phenotypically-distinct neuronal populations between the left and right hemispheres. Given the right amygdala's sensitivity to emotional stress compared to the left, we hypothesized that there would be more inhibitory, PV-containing neurons in the left BLC compared with the right. Therefore, the goal of the present experiments was to acquire an estimate of the number of PV-positive neurons in subdivisions of the left and right BLC using unbiased stereology. In addition, volume comparisons were made between the left and right BLC and their subdivisions.

2. Results

2.1 Hemispheric differences in the number of parvalbumin-positive neurons in the BLC

The optical fractionator method was used to determine an unbiased estimate of the number of PV-positive neurons (Figure 2A) in subdivisions of the left and right BLC (Figures 2B and C). Two-way repeated measures ANOVA revealed significant effects of hemisphere ($F_{1,6}$ = 21.73; P = 0.0035) and subdivision ($F_{2,6}$ = 5.611; P = 0.0423) on the unbiased estimate of PV-positive neurons in the basolateral amygdala (Figure 2C). Bonferonni's multiple comparison test revealed that the left posterior basolateral subdivision contained more PV-positive neurons than the right (Figure 2C; P < 0.01). There was a significant effect of subdivision ($F_{2,6}$ = 9.175; P = 0.0150), but not hemisphere; ($F_{1,6}$ = 1.891; P = 0.2183), on the number of PV-positive neurons in the lateral amygdala (Figure 2B).

2.2 Hemispheric differences in volume of the lateral amygdala

In addition to unbiased estimates of phenotypic populations, unbiased estimates of volume for each subdivision were acquired in the lateral (Figure 2D) and basolateral amygdala (Figure 2E). A significant effect of subdivision was found in the lateral ($F_{2,6}$ = 8.964; P = 0.0158) and basolateral amygdala ($F_{2,6}$ = 8.485; P = 0.0178) as well as a significant effect of hemisphere ($F_{1,6}$ = 12.82; P = 0.0116) in the lateral amygdala.

2.3 Hemispheric differences in density of PV-positive neurons in the BLC

Using the unbiased counts for PV-positive neurons and volume, density of PV-positive neurons for each subdivision was calculated in the lateral (Figure 2F) and basolateral

amygdala (Figure 2G). A significant effect of hemisphere was measured in the basolateral amygdala ($F_{1,6}$ = 9.645; P = 0.0210). Like the neuronal count data, the left basolateral amygdala had a greater density of PV-positive neurons then the right hemisphere.

3. Discussion

In the current study, we utilized the optical fractionator method of unbiased stereology to acquire accurate estimates of the number of PV-positive neurons in the left and right hemispheres in subdivisions of the basolateral amygdalar complex. The total number of PV-positive neurons was acquired in the lateral dorsolateral, lateral ventrolateral, lateral ventromedial (comprising the lateral amygdala), and the basolateral anterior, basolateral posterior, basolateral ventral (comprising the basolateral amygdala). In addition, volume and density estimates were calculated for each subdivision. The left basolateral amygdala possessed a higher estimated number of PV-positive neurons than the right, and this difference was significant in the posterior portion of the basolateral amygdala. The volume measurements of the left lateral amygdala were also larger than the right lateral amygdala. Furthermore, an overall higher density of PV-positive neurons was observed in the left compared to the right BLC. This hemispheric density difference was only seen in the basolateral region with no statistical differences in the number of PV-positive neurons or PV-neuronal density in the lateral amygdala.

The purpose of this experiment was to investigate anatomical differences in the left and right BLC. The original hypothesis, based on the right amygdala's sensitivity to emotional stress, was there would be more inhibitory control in the left amygdala than the right. The data presented here supports this hypothesis, as there were significantly more PV-positive neurons and a higher density of PV-positive neurons in the left basolateral amygdala than the right. PV-positive neurons have been demonstrated to exert inhibitory control over local neurons (McDonald et al., 2005) through burst- and stutter-firing patterns (Rainnie et al., 2006). Greater inhibitory control could explain the enhanced activation of the right over the left amygdala in response to nociception (Ji and Neugebauer, 2009). While it was demonstrated that PV-positive neurons do not fire in synchrony with noxious stimuli, the tight regulation of CAMKII projection neurons by PV-positive neuron GABA release suggests the possibility that instead of static activity, dynamic activity via PV-neurons of the BLC underpins the response to noxious stimuli (Bienvenu et al., 2012). Given PV neuron regulation of glutamatergic pyramidal neurons in the BLC, this greater inhibitory control on the left compared with the right could also explain the differential effects of unilateral glutamate (NMDA) antagonists on long-lasting responses following predator stress (Adamec et al., 2005; Adamec et al., 1999).

The higher number of PV-positive neurons in the left basolateral amygdala could also result in decreased long-term potentiation (LTP) to certain stimuli. This is supported by Adamec et al. (2005) findings that showed shorter potentiation in efferent pathways from the left amygdala in response to predator stress. Orman and Stewart (2007) demonstrated that PKC levels were higher in the left amygdala than the right. Since PKC functions to inhibit G-protein inwardly rectifying K+ channels (Mao et al., 2004), this may also provide a mechanism for decreased LTP in the left amygdala than the right. LTP has been implicated

in amygdala-mediated fear conditioning (Malenka and Bear, 2004). Further studies are needed to clarify the underlying properties that explain lateralization of LTP between the left and right amygdala, and the specific role of PV-containing neurons in these lateralized functional responses. Using unbiased stereology, we show here that there are significantly more PV-positive neurons in the left basolateral amygdala than the right. This finding may underpin the unilateral differences in behavior as well as the reduced long-term potentiation of the left versus the right amygdala. A confounding aspect of these propositions is that it is predicated on numerous studies that did not differentiate between the left and right amygdala when initially describing the properties of the neurons. These include studies investigating the electrophysiological properties of the neurons as well as co-localization of neuropeptides or other markers with neurotransmitters. Furthermore, many behavior studies use bilateral manipulation of the amygdala, which limits the ability to differentiate between hemispheric lateralization of function. On this note, it would also be useful to compare the functional connectivity between the left and right amygdala. Similarly, many studies do not differentiate between the subdivisions of the BLC. The study presented here concludes that there are more PV-positive neurons in the left basolateral amygdala than the right with highly significant differences in number in the basolateral posterior subdivision; however, any comparison with prior studies that demonstrated hemispheric lateralization of function must take into account the specificity of anterior/posterior and other subdivisions of the amygdala. Taken together, this study supports other studies that demonstrate striking differences in the function and anatomical properties of the left versus the right amygdala, and could help explain the hemispheric differences in amygdala activation seen in many human studies (Baas et al., 2004; Simons et al., 2014). Understanding the lateralization of function of the amygdala could impact the development of novel strategies for targeting amygdalar dysfunction in disease states.

4. Experimental Procedures

4.1 Animals

Five male Long-Evans rats (Harlan Laboratories, Indianapolis, IN), weighing 250–300g were single-housed in an environmentally controlled animal facility on a 12:12 h light: dark cycle with lights on at 0700 hours. Purina rat chow and water were available *ad libitum*. All experiments were conducted during the light phase, beginning at least 2 hours after light phase onset. Animals were housed in an animal facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Animal care and use procedures were carried out in accordance with protocols written under the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the University of South Carolina.

4.2 Perfusion and immunohistochemistry

Rats were placed under deep isoflurane anesthesia and transcardially perfused with 0.1M phosphate buffered saline followed by 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). The brains were removed, post-fixed overnight and transferred to 30% sucrose solution in 0.1 M phosphate buffer for cryoprotection (Butler et al., 2011; Butler et al., 2012; Butler

et al., 2016). Prior to sectioning, a diagonal cut was made into the cortex throughout the length of the brains on the right side so that the hemispheres could be differentiated after staining. Three brains were cut into coronal sections on a microtome at a thickness of 45 microns and divided evenly into three serial sets for PV-staining. The sections were then stored at −20°C in anti-freezing solution (30% sucrose and 30% ethylene glycol in 0.1M phosphate buffer) until they were processed for immunohistochemistry.

Immunohistochemistry from amygdalar sections for all rats was performed at the same time as previously described (Butler et al., 2011). Sections were incubated in mouse primary antisera directed against PV (1:8000; Sigma-Aldrich, St. Louis, MO) at 4°C for 48 hours, followed by incubation with unlabeled donkey anti-mouse secondary antibodies (1:100; Jackson ImmunoResearch Laboratories Inc.) for 2 hours at room temperature and finally with mouse peroxidase-anti-peroxidase (1:250; Covance, Florida). PV antibodies had been previously verified (Reznikov et al., 2008). As described in Reznikov *et al.* (2008), the antiserum for PV was derived from a PARV-19 hybridoma produced by fusion of mouse myeloma cells and splenocytes from an immunized mouse (manufacturer's information). Purified frog muscle parvalbumin was used as the immunogen. Previous characterization of this antibody indicated that it reacts with a 12-kDa PV protein, but not with other members of the calcium-binding protein family such as calmodulin, intestinal calcium-binding protein, S100A2, and calcyclin (manufacturer's information). The labeling of neuronal bodies produced by this antibody was consistent with previous studies (Butler et al., 2011; Reznikov et al., 2008) as well as with studies utilizing other PV antisera (McDonald and Betette, 2001). Immunoreactivity for PV was visualized by developing the sections in plain diaminobenzidine solution with 3.0% hydrogen peroxide, yielding a brown reaction product confined to the cytoplasm of immunoreactive cells with these markers. After standard mounting, sections were dehydrated and coverslipped using Permount mounting medium.

4.3 Optical Fractionator Method

Unbiased stereology affords us an opportunity to acquire an accurate estimate of the total number of labeled neurons over the 3D space of the region of interest (ROI) (West, 2012). The optical fractionator method is a uniform systematic sampling of 3D probes known as optical dissectors (West et al., 1991). The StereoInvestigator program (version 9.0, MBF Bioscience; Williston, VT) was used to generate the unbiased counts of PV-positive neurons and total volume of the ROI using the optical fractionator method. We determined with a preliminary experiment that due to the sparse staining of PV, the optical disector needed to be at 60 × 60 microns – the maximum allowed by the StereoInvestigator program. These dimensions were determined to be sufficient to count between 1 and 3 cells per counting frame. As stated in the previous section, every third section was stained for PV throughout the rostro-caudal amygdala for three rat brains. The sections lost between 50–55% of their thickness following dehydration. All sections maintained a thickness of at least 20 microns for each counting frame. Section thickness was also determined at each individual counting frame. The upper and lower guard zones were each 2 microns leaving a counting zone of at least 16 microns through which neurons which came into focus were counted. The counting step was determined automatically by the StereoInvestigator program. PV-stained neurons which touched the red line of the optical disector were not counted to avoid double-counting

of single neurons whereas neurons which touched only the green line were counted. All counts were done under 100× magnification after the ROI was outlined at 2× magnification. The number estimates were determined by using number weighted for section thickness. Volume was automatically determined by the StereoInvestigator program. The left and right BLC was subdivided into lateral dorsolateral, lateral ventrolateral, lateral ventromedial, basolateral anterior, basolateral posterior and basolateral ventral (Figure 1). The subdivisions of the BLC were determined by a trained investigator and were based on the Paxinos and Watson rat brain atlas (1997).

4.4 Data analysis

For each subdivision in the left and right BLC, total number and volume (in mm^3) were calculated by the StereoInvestigator program. The coefficient of error was also determined using the StereoInvestigator program. For all estimates, coefficients of error ≤ 0.10 were accepted; as a result, 2 of the 5 data points for each data set were removed resulting in a final group size of 3. While low, the group sizes are sufficient based on previous stereology publications (Boulanger and Messier, 2017; West, 2013). The data was divided into left and right subdivisions of the lateral amygdala (lateral dorsolateral, lateral ventrolateral, and lateral ventromedial) and basolateral amygdala (basolateral anterior, basolateral posterior and basolateral ventral) for separate analyses. All data were analyzed with 2-way (hemisphere and subdivision) repeated measures analysis of variance (ANOVA) using GraphPad Prism® (version 6.07) followed by Bonferonni's multiple comparisons test. Significance level was set at alpha = 0.05.

Acknowledgments

This work was funded by a grant from NIMH RO1 MH063344 to MAW and JRF, a Young Investigator Award from the Brain & Behavior Research Foundation to RKB, a VA Merit Award funding to MAW (1101 BX001374; 1101BX002085 and 1101BX001804), and a Science Undergraduate Research Fellowship and Magellan Fellowship from University of South Carolina to EMO.

References

Adamec R, Walling S, Burton P. Long-lasting, selective, anxiogenic effects of feline predator stress in mice. Physiol Behav. 2004; 83:401–10. [PubMed: 15581662]

Adamec R, Blundell J, Burton P. Role of NMDA receptors in the lateralized potentiation of amygdala afferent and efferent neural transmission produced by predator stress. Physiol Behav. 2005; 86:75–91. [PubMed: 16102787]

Adamec RE. Evidence that limbic neural plasticity in the right hemisphere mediates partial kindling induced lasting increases in anxiety-like behavior: effects of low frequency stimulation (quenching?) on long term potentiation of amygdala efferents and behavior following kindling. Brain Res. 1999; 839:133–52. [PubMed: 10482807]

Adamec RE, Burton P, Shallow T, Budgell J. NMDA receptors mediate lasting increases in anxiety-like behavior produced by the stress of predator exposure–implications for anxiety associated with posttraumatic stress disorder. Physiol Behav. 1999; 65:723–37. [PubMed: 10073474]

Adolphs R, Tranel D, Denburg N. Impaired emotional declarative memory following unilateral amygdala damage. Learn Mem. 2000; 7:180–6. [PubMed: 10837507]

Augustine, JR. Human Neuroanatomy. Vol. 2. Wiley-Blackwell; 2017.

Baas D, Aleman A, Kahn RS. Lateralization of amygdala activation: a systematic review of functional neuroimaging studies. Brain Res Brain Res Rev. 2004; 45:96–103. [PubMed: 15145620]

Bienvenu TC, Busti D, Magill PJ, Ferraguti F, Capogna M. Cell-type-specific recruitment of amygdala interneurons to hippocampal theta rhythm and noxious stimuli in vivo. Neuron. 2012; 74:1059–1074. [PubMed: 22726836]

Boulanger JJ, Messier C. Unbiased stereological analysis of the fate of oligodendrocyte progenitor cells in the adult mouse brain and effect of reference memory training. Behav Brain Res. 2017; 329:127–139. [PubMed: 28442356]

Buchanan TW, Denburg NL, Tranel D, Adolphs R. Verbal and nonverbal emotional memory following unilateral amygdala damage. Learn Mem. 2001; 8:326–35. [PubMed: 11773432]

Butler RK, Sharko AC, Oliver EM, Brito-Vargas P, Kaigler KF, Fadel JR, Wilson MA. Activation of phenotypically-distinct neuronal subpopulations of the rat amygdala following exposure to predator odor. Neuroscience. 2011; 175:133–44. [PubMed: 21146592]

Butler RK, White LC, Frederick-Duus D, Kaigler KF, Fadel JR, Wilson MA. Comparison of the activation of somatostatin- and neuropeptide Y-containing neuronal populations of the rat amygdala following two different anxiogenic stressors. Exp Neurol. 2012; 238:52–63. [PubMed: 22917777]

Butler RK, Oliver EM, Sharko AC, Parilla-Carrero J, Kaigler KF, Fadel JR, Wilson MA. Activation of corticotropin releasing factor-containing neurons in the rat central amygdala and bed nucleus of the stria terminalis following exposure to two different anxiogenic stressors. Behav Brain Res. 2016; 304:92–101. [PubMed: 26821289]

Celio MR. Parvalbumin in most gamma-aminobutyric acid-containing neurons of the rat cerebral cortex. Science. 1986; 231:995–997. [PubMed: 3945815]

Celio MR. Calbindin D-28k and parvalbumin in the rat nervous system. Neuroscience. 1990; 35:375–475. [PubMed: 2199841]

Cooney RE, Atlas LY, Joormann J, Eugene F, Gotlib IH. Amygdala activation in the processing of neutral faces in social anxiety disorder: is neutral really neutral. Psychiatry Res. 2006; 148:55–9. [PubMed: 17030117]

Ji G, Neugebauer V. Hemispheric lateralization of pain processing by amygdala neurons. J Neurophysiol. 2009; 102:2253–64. [PubMed: 19625541]

Kemppainen S, Pitkanen A. Distribution of parvalbumin, calretinin, and calbindin-D(28k) immunoreactivity in the rat amygdaloid complex and colocalization with gamma-aminobutyric acid. J Comp Neurol. 2000; 426:441–467. [PubMed: 10992249]

Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. Neuron. 2004; 44:5–21. [PubMed: 15450156]

Mao J, Wang X, Chen F, Wang R, Rojas A, Shi Y, Piao H, Jiang C. Molecular basis for the inhibition of G protein-coupled inward rectifier K(+) channels by protein kinase C. Proc Natl Acad Sci U S A. 2004; 101:1087–92. [PubMed: 14732702]

Mascagni F, McDonald AJ. Parvalbumin-immunoreactive neurons and GABAergic neurons of the basal forebrain project to the rat basolateral amygdala. Neuroscience. 2009; 160:805–812. [PubMed: 19285116]

McDonald AJ, Betette RL. Parvalbumin-containing neurons in the rat basolateral amygdala: morphology and co-localization of Calbindin-D(28k). Neuroscience. 2001; 102:413–425. [PubMed: 11166127]

McDonald AJ, Mascagni F. Immunohistochemical characterization of somatostatin containing interneurons in the rat basolateral amygdala. Brain Res. 2002; 943:237–244. [PubMed: 12101046]

McDonald AJ, Mascagni F, Mania I, Rainnie DG. Evidence for a perisomatic innervation of parvalbumin-containing interneurons by individual pyramidal cells in the basolateral amygdala. Brain Res. 2005; 1035:32–40. [PubMed: 15713274]

McDonald AJ, Mascagni F, Zaric V. Subpopulations of somatostatin-immunoreactive non-pyramidal neurons in the amygdala and adjacent external capsule project to the basal forebrain: evidence for the existence of GABAergic projection neurons in the cortical nuclei and basolateral nuclear complex. Front Neural Circuits. 2012; 6:46. [PubMed: 22837739]

Muller JF, Mascagni F, McDonald AJ. Pyramidal cells of the rat basolateral amygdala: synaptology and innervation by parvalbumin-immunoreactive interneurons. J Comp Neurol. 2006; 494:635–650. [PubMed: 16374802]

Orman R, Stewart M. Hemispheric differences in protein kinase C betaII levels in the rat amygdala: baseline asymmetry and lateralized changes associated with cue and context in a classical fear conditioning paradigm. Neuroscience. 2007; 144:797–807. [PubMed: 17118565]

Patestas, MA., Gartner, LP. A Textbook of Neuroanatomy. Vol. 2. John Wiley & Sons; 2016.

Paxinos, G., Watson, C. The Rat Brain in Stereotaxic Coordinates. Vol. 3. Academic Press; New York: 1997.

Rainnie DG, Mania I, Mascagni F, McDonald AJ. Physiological and morphological characterization of parvalbumin-containing interneurons of the rat basolateral amygdala. J Comp Neurol. 2006; 498:142–61. [PubMed: 16856165]

Reznikov LR, Reagan LP, Fadel JR. Activation of phenotypically distinct neuronal subpopulations in the anterior subdivision of the rat basolateral amygdala following acute and repeated stress. J Comp Neurol. 2008; 508:458–472. [PubMed: 18335544]

Sah P, Faber ES, Lopez De Armentia M, Power J. The amygdaloid complex: anatomy and physiology. Physiol Rev. 2003; 83:803–34. [PubMed: 12843409]

Simons LE, Moulton EA, Linnman C, Carpino E, Becerra L, Borsook D. The human amygdala and pain: evidence from neuroimaging. Hum Brain Mapp. 2014; 35:527–38. [PubMed: 23097300]

Sorvari H, Soininen H, Paljarvi L, Karkola K, Pitkanen A. Distribution of parvalbumin-immunoreactive cells and fibers in the human amygdaloid complex. J Comp Neurol. 1995; 360:185–212. [PubMed: 8522643]

West MJ, Slomianka L, Gundersen HJ. Unbiased stereological estimation of the total number of neurons in thesubdivisions of the rat hippocampus using the optical fractionator. Anat Rec. 1991; 231:482–97. [PubMed: 1793176]

West MJ. The precision of estimates in stereological analyses. Cold Spring Harb Protoc. 2012; 2012:937–949. [PubMed: 22949720]

West MJ. Optimizing the sampling scheme for a stereological study: how many individuals, sections, and probes should be used. Cold Spring Harb Protoc. 2013; 2013:521–32. [PubMed: 23734015]

Wilson MA, Grillo CA, Fadel JR, Reagan LP. Stress as a one-armed bandit: Differential effects of stress paradigms on the morphology, neurochemistry and behavior in the rodent amygdala. Neurobiol Stress. 2015; 1:195–208. [PubMed: 26844236]

Highlights

The left basolateral amygdala contains a significantly higher number and density of parvalbumin-positive neurons than the right basolateral amygdala

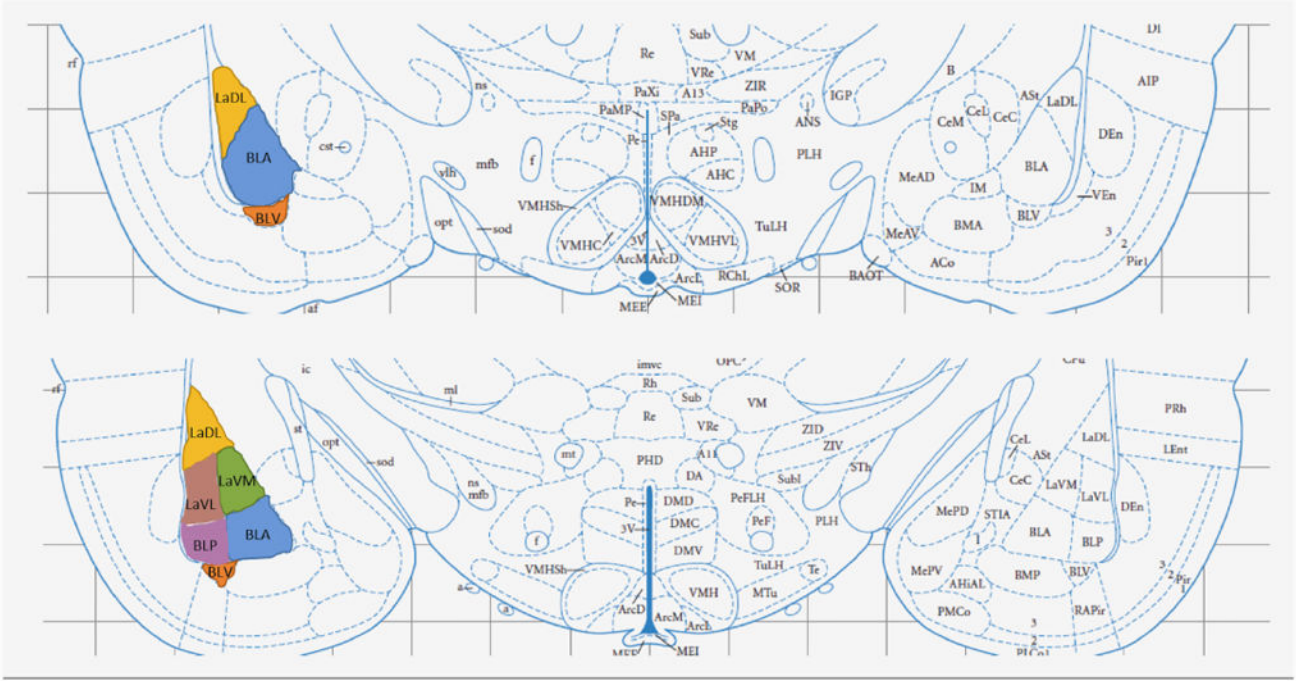

Figure 1.

Using unbiased stereology to determine the differences in total number of parvalbumin (PV)-positive neurons between the left and right basolateral amygdala complex. The optical disector method was used to acquire an unbiased estimate of PV-positive neurons. Anterior (top) and posterior (bottom) portions of subdivisions within the basolateral amygdala complex in which PV-positive neurons were counted. Abbreviations: LaDL = dorsolateral part of the lateral amygdaloid nucleus, LaVL = ventrolateral part of the lateral amygdaloid nucleus, LaVM = ventromedial part of the lateral amygdaloid nucleus, BLA = anterior basolateral amygdaloid nucleus, BLP = posterior basolateral amygdaloid nucleus, BLV = ventral basolateral amygdaloid nucleus; based on the Paxinos and Watson rat brain atlas (1997).

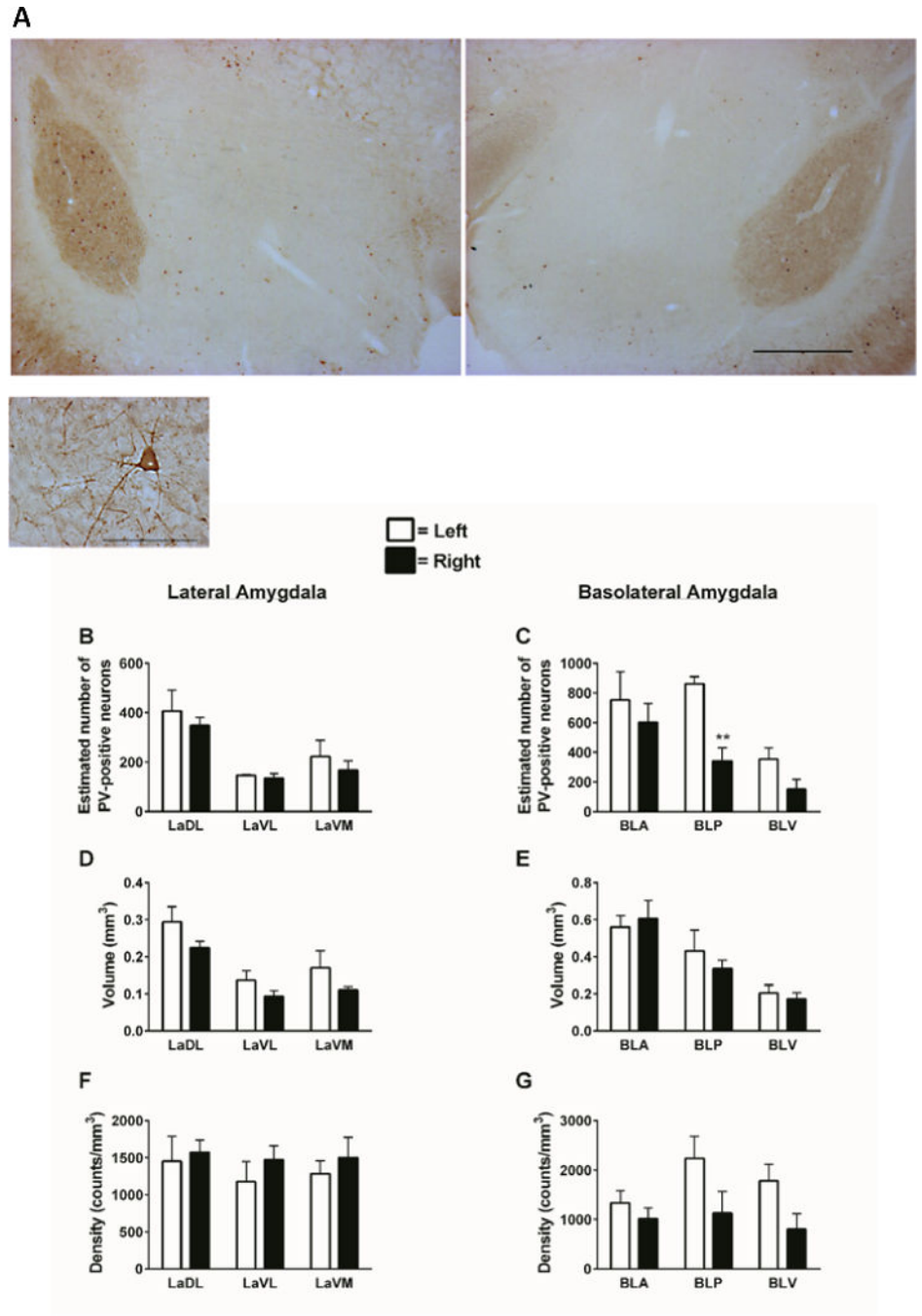

Figure 2.

Unbiased estimates of the number of parvalbumin (PV)-positive neurons in the left and right basolateral amygdala complex. (A) Top: representative photomicrograph of the left and right hemispheres of the basolateral amygdala complex which had been immunostained for PV (2× magnification; scale bar = ~500 μm). Bottom: representative photomicrograph of a single PV-stained neuron (40× magnification; scale bar = 50 μm). Unbiased estimates of the total number of PV-positive neurons were acquired in subdivisions of the lateral (B) and basolateral (C) amygdala. In addition, unbiased estimates of volume were acquired in

subdivisions of the lateral (D) and basolateral (E) amygdala. From the measurements acquired for counts and volume, the density of PV-positive neurons were measured in the lateral (F) and basolateral (G) amygdala. A statistically significant difference was measured between the left and right basolateral amygdala with the left basolateral posterior subdivisions containing more PV-positive neurons than the right counterpart. There was also a greater density of PV-positive neurons in the left compared to the right basolateral amygdala. Data are means ± SEM. **P<0.01. N=3.