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Research report

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

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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 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 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

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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., 1999). 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, 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 (Fig. 2A) in subdivisions of the left and right BLC (Fig. 2B and C). Two-way repeated measures ANOVA revealed significant effects of hemisphere ($F_{1,6} = 21.73$; P = .0035) and subdivision ($F_{2,6} = 5.611$; P = .0423) on the unbiased estimate of PV-positive neurons in the basolateral amygdala (Fig. 2C). Bonferonni's multiple comparison test revealed that the left posterior basolateral subdivision contained more PV-positive neurons than the right (Fig. 2C; P < .01). There was a significant effect of subdivision ($F_{2,6} = 9.175$; P = .0150), but not hemisphere; ($F_{1,6} = 1.891$; P = .2183), on the number of PV-positive neurons in the lateral amygdala (Fig. 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 (Fig. 2D) and basolateral amygdala (Fig. 2E). A significant effect of subdivision was found in the lateral ($F_{2,6} = 8.964$; P = .0158) and basolateral amygdala ($F_{2,6} = 8.485$; P = .0178) as well as a significant effect of hemisphere ($F_{1,6} = 12.82$; P = .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 (Fig. 2F) and basolateral amygdala (Fig. 2G). A significant effect of hemisphere was measured in the basolateral amygdala ($F_{1,6} = 9.645$; P = .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