Published in final edited form as:

Neurosci Lett. 2007 July 5; 422(1): 13–17. doi:10.1016/j.neulet.2007.05.041.

OLFACTORY SYSTEM MODULATION OF HIPPOCAMPAL CELL DEATH

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Abstract

The hippocampal dentate gyrus is a major recipient of olfactory input in rodents, via connections from the olfactory (piriform) cortex and the olfactory bulb to the entorhinal cortex. Given this connectivity and the known role of activity in dentate gyrus granule cell survival, the present experiment examined the immediate effects of loss of olfactory input to the hippocampus on apoptosis. Adult rats underwent unilateral or bilateral olfactory bulb ablations (OBX), and allowed to recover 24–72 hours before the piriform cortex and hippocampal dentate gyrus were processed for terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling [TUNEL] of apoptotic cells. OBX transiently increased TUNEL-positive cells in the ipsilateral piriform cortex and dentate gyrus. Increased TUNEL-labeling was apparent within 24 hrs in both structures, but was more extensive and prolonged in piriform cortex. The results suggest a trans-synaptic regulation of cell survival through at least two synapses.

Keywords

apoptosis; olfactory bulb lesions; piriform cortex; memory; TUNEL; neurogenesis

INTRODUCTION

Neural survival in the central nervous system is often activity dependent. Both over-activation, as in excitotoxicity, and loss of afferent input, as in sensory deprivation or degenerative disease can lead to cell death through necrosis or apoptosis. Transsynaptic mediators of neuronal survival range from activity-dependent release of neurotrophic factors which can maintain connectivity and survival of recipient cells, to classic amino acid neurotransmitters such as glutamate, which if released at high levels can initiate a cascade of events in directly targeted or nearby cells leading to their death [3,22].

The central olfactory pathway appears to be a prime example of these processes. Olfactory information travels from the olfactory bulb to olfactory cortical areas including the piriform cortex, and then directly to the entorhinal cortex which is the major afferent to the hippocampal dentate gyrus [11,16]. Two regions uniformly agreed to display continual neurogenesis through life are the olfactory bulb and the hippocampal dentate gyrus [1,2,15,18]. In both structures, survival of adult generated neurons is dependent on their being incorporated into an active

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circuit [4,9,18,23]. For example, exposure to specific odors enhances the survival of adult-born granule cells within spatial regions of the olfactory bulb activated by those odors [23]. Olfactory deprivation, in contrast, enhances olfactory bulb granule cell apoptosis [21]. Similarly, survival of dentate gyrus granule cells born in adults is enhanced in animals actively engaged in hippocampal dependent tasks [9,25]. Interestingly, survival of a specific population of pyramidal-like neurons in the piriform cortex is also exquisitely sensitive to afferent input, although this population does not appear to display adult neurogenesis. Semilunar pyramidal cells in Layer IIa of the piriform cortex undergo rapid apoptosis following either loss of afferent input due to bullectomy [17,19] or decreased afferent activity due to sensory deprivation [17]. Death of semilunar cells in the bullectomy model is not mediated by axotomy, since they do not send axons back to the olfactory bulb [5,8,10,13], but rather appears to be mediated by a sudden surge of glutamate release from isolated mitral cell axon terminals within piriform cortex and a resulting glutamate-mediated nitric oxide initiated apoptotic cascade [14,30].

Given the close anatomical relationship between the hippocampal dentate gyrus and the olfactory system [16], and critical role the hippocampus plays in odor memory [7,27], the present experiment directly examined the effects of unilateral or bilateral olfactory bullectomy on apoptosis in the dentate gyrus. The time course and extent of apoptosis (TUNEL-labeling) were compared between the piriform cortex and hippocampus. The results demonstrate a rapid, transient increase in apoptosis in the dentate gyrus ipsilateral to the removed olfactory bulb, and suggest that olfaction is an important contributor to hippocampal cell survival.

METHODS

Male Long-Evans hooded rats born in the colony at the University of Oklahoma were used as subjects. They were maintained on a 12 hr light/dark cycle and had food and water available ad lib. Experimental procedures and animal care conformed to US Public Health Service Policy on Human Care and Use of Laboratory Animals and were approved by the University of Oklahoma Institutional Animal Care and Use Committee.

For surgical olfactory bulb ablation, rats (200–300g) were anesthetized with isoflurane and placed in a stereotaxic apparatus. Following a craniotomy over the olfactory bulb, unilateral or bilateral olfactory bulb ablations were performed with aspiration through an 18 ga blunted stainless steel needle, as previously described [17]. Following aspiration, the craniotomy was filled with bone wax and the scalp sutured close. Rats received unilateral (n = 4) or bilateral (n = 4) olfactory bulb aspiration lesions or sham (craniotomy only) unilateral (n = 5) or bilateral (n = 7) lesions and allowed to recover for 24 hr. An additional group of rats received unilateral olfactory bulb aspiration lesions and allowed to recover for 36 hrs (n = 4), 48 hrs (n = 4) or 72 hrs (n = 4). Following recovery, animals were overdosed and the brain rapidly removed and frozen in 2-methylbutane (–45°C) and maintained at –70°C until processing for (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling [TUNEL]).

Coronal sections (20 μ) were taken through the anterior piriform cortex (approximately centered around 1.0 mm anterior to Bregma) and dorsal hippocampus (approximately centered around 3.0 mm posterior to Bregma), with at least 5 sections through each area and 60–100 μ between sections. Sections were placed on slides, fixed with 4% paraformaldehyde in 0.1 M phosphate buffered saline. Details of the TUNEL reaction are provided in Leung & Wilson [17]. Sections were incubated in TUNEL reaction mixture according to kit instructions (Roche Molecular Biochemicals) at 37 °C for 1 h in a humidified chamber. The TUNEL reaction mixture was prepared with a 1:2 dilution of the enzyme solution, which was mixed with the label solution just before use. TUNEL labeling was visualized with diaminobenzidine chromogen, and slides coverslipped and coded for subsequent blind cell counts. For this study, labeled cell types were not identified as to glial or neuronal origin, though previous studies

have demonstrated the majority of piriform cortical cells undergoing apoptosis after bulbectomy are semilunar pyramidal neurons [5].

Darkly and uniformly stained cell nuclei were counted within individual sections through the full extent of Layer II of the anterior piriform cortex and in the dentate gyrus granule cell layer plus granular/hilar boundary, bilaterally in all animals. Cell counting was performed with a Olympus BH-2 microscope and camera lucida. Cell counts were expressed as number of labeled cells/section and averaged across at least 5 sections for each hemisphere in each animal. Statistical comparisons across experimental conditions and survival duration were performed with ANOVA's and post-hoc tests.

RESULTS

As shown in Fig. 1, olfactory bulbectomy resulted in a significant increase in TUNEL labeled cells within 24 hr in the anterior piriform cortex Layer II. Unilateral OBX induced ipsilateral apoptosis only, while bilateral OBX induced bilateral apoptosis. ANOVA analysis at the 24 hr survival time point revealed a significant effect of OBX ($F(7,32) = 58.74$, $p < 0.001$). Post-hoc tests revealed TUNEL labeled cell counts were significantly higher in piriform cortex ipsilateral to the OBX in unilateral and bilateral lesioned rats compared to contralateral cortex and control rats ($p < 0.05$). TUNEL-labeled cell counts remained significantly elevated for at least 48 hrs after unilateral OBX (side $\times$ survival duration interaction ANOVA, $F(3,12) = 5.08$, $p < 0.02$).

As shown in Fig. 2, olfactory bulbectomy also resulted in a significant increase in TUNEL labeled cells within 24 hr in the dentate gyrus, although the magnitude of the effect was smaller than that observed in the piriform cortex. Unilateral OBX induced ipsilateral apoptosis only, while bilateral OBX induced bilateral apoptosis. ANOVA analysis at the 24 hr survival time point revealed a significant effect of OBX ($F(7,32) = 3.66$, $p < 0.01$). Post-hoc tests revealed TUNEL labeled cell counts were significantly higher in dentate gyrus ipsilateral to the OBX in unilateral and bilateral lesioned rats compared to contralateral cortex and control rats ($p < 0.05$). In contrast to the piriform cortex, TUNEL-labeled cell counts returned to baseline within 36 hrs after unilateral OBX (side $\times$ survival duration interaction ANOVA, $F(3,12) = 5.22$, $p < 0.02$).

A within animal comparison of TUNEL positive cell counts at 24 hrs post OBX ipsilateral to the lesion showed a positive correlation of $r = 0.33$ ($n=12$) between piriform cortex and dentate gyrus. Thus within animals, as cell death increased in one area there was a tendency for an increase in the other.

DISCUSSION

These results demonstrate that input from ipsilateral olfactory structures plays a role in hippocampal dentate cell death, in addition to the roles previously demonstrated for exploration and learning [9,25], exercise [4,6,28] and hormones and stress [20]. The fact that the effects were limited to the ipsilateral hemisphere in unilateral OBX animals argues against a generalized or widespread system degeneration. Increased apoptosis was apparent within 24 hrs following OBX in both the piriform cortex and dentate gyrus. Interestingly, while level of cell death was correlated in the two structures, the wave of labeling in the piriform cortex outlasted that seen in the dentate gyrus, suggesting that the loss of piriform cells itself may not be the sole driving factor in dentate gyrus cell loss.

The results in the piriform cortex confirm previous work showing that loss of afferent input, either from bulbectomy or sensory deprivation induces a transient wave of apoptosis ipsilateral to the afferent loss [5,8,17]. Recent evidence suggests that OBX causes an uncontrolled release

of glutamate from severed mitral cell axons, which via a novel AMPA receptor mechanism [30], causes nitric oxide release from local interneurons [14] to induce an apoptotic cascade in semilunar cells [19]. Semilunar cell loss due to sensory deprivation may be due to a different mechanism since its onset and time course are substantially delayed compared to OBX [17]. The mechanisms leading to the observed ipsilateral apoptosis in the dentate gyrus, however, are unclear. For example, while the major output from the olfactory bulb is ipsilateral, the olfactory cortex and thus presumably hippocampus, have access to bilateral input [29]. The rapid time course of cell death in dentate gyrus may argue against loss of neurotrophic factors as an immediate cause. A small subset of mitral cells project directly to the entorhinal cortex [24], thus a transsynaptic excitotoxicity, similar to that in the piriform cortex, could contribute to the cell loss in dentate gyrus.

Interestingly, bilateral OBX is also currently utilized as a model for depression in rodents [26]. Recent work has demonstrated that there is a reduction in dentate granule cell number and a decrease in granule cell proliferation 6 weeks after the lesions [12]. There was no significant change in TUNEL-positive cells 6 weeks post-lesion, in accordance with the present findings of a very transient wave of dentate cell death. Together with the present results, this suggests that OBX induces a trans-synaptic reduction in hippocampal dentate gyrus cell survival involving both a rapid wave of apoptosis (present results) and a subsequent and prolonged down-regulation of cell proliferation [12]. They further demonstrate the long range effects that local damage, disease or degeneration can have throughout large scale central circuits.

Acknowledgments

This work was supported by a grant from NIDCD to DAW. The authors would like to thank Zack Bowen for assistance with blind cell counts.

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Figure 1.

Olfactory bulbectomy induced a rapid and profound increase in TUNEL-labeled cells in the anterior piriform cortex layer II ipsilateral to the lesion. **(A)** Representative sections of TUNEL-labeled cells in the ipsilateral and contralateral anterior piriform cortex 24 hrs after bulbectomy. Cortical layers are marked. **(B)** The enhanced apoptosis was expressed ipsilateral to OBX in both unilateral and bilateral OBX animals at 24 hrs post lesion. **(C)** This enhanced apoptosis was apparent within 24 hr of the lesion and lasted at least 48 hr. No bilateral OBX or control animals were tested at the 36–72 hr time points.

Figure 2.

Olfactory bulbectomy induced a rapid increase in TUNEL-labeled cells in the dentate gyrus ipsilateral to the lesion. (**TOP**) Representative sections of TUNEL-labeled cells in the ipsilateral and contralateral dentate gyrus 24 hrs after bulbectomy. Arrows indicate TUNEL labeled nuclei. Granule cell layer (gr) and dentate hilus (h) are marked. (**B**) The enhanced apoptosis was expressed ipsilateral to OBX in both unilateral and bilateral OBX animals at 24 hrs post lesion. (**C**) This enhanced apoptosis was apparent within 24 hr of the lesion and returned to baseline within 36 hr, in contrast to the more prolonged effect observed in piriform cortex. No bilateral OBX or control animals were tested at the 36–72 hr time points.