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AGING-RELATED ALTERATIONS IN OREXIN/HYPOCRETIN MODULATION OF SEPTO-HIPPOCAMPAL AMINO ACID NEUROTRANSMISSION

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Abstract—GABAergic neurons of the medial septum of the basal forebrain make up a substantial portion of the septo-hippocampal pathway fibers, and are known to modulate hippocampal amino acid neurotransmission and support cognitive function. Importantly, these neurons are also implicated in age-related cognitive decline. Hypothalamic orexin/hypocretin neurons innervate and modulate the activity of these basal forebrain neurons and also provide direct inputs to the hippocampus. However, the precise role of orexin inputs in modulating hippocampal amino acid neurotransmission—as well as how these interactions are altered in aging—has not been defined. Here, orexin A (OxA) was administered to CA1 and the medial septum of young (3–4 months) and aged (27–29 months) Fisher 344 Brown Norway rats, and hippocampal GABA and glutamate efflux was analyzed by *in vivo* microdialysis. Following CA1 infusion of OxA, extracellular GABA and glutamate efflux was increased, but the magnitude of orexin-mediated efflux was not altered as a function of age. However, medial septum infusion of OxA did not impact hippocampal efflux in young rats, while aged rats exhibited a significant enhancement in GABA and glutamate efflux compared to young counterparts. Furthermore, immunohistochemical characterization of the medial septum revealed a significant decrease in parvalbumin (PV)-positive cell bodies in aged animals, and a significant reduction in orexin fiber innervation to the remaining GABAergic cells within the septum, while orexin innervation to the hippocampus was unaltered by the aging process. These findings indicate that: (1) OxA directly modulates hippocampal amino acid neurotransmission in young animals, (2) Aged animals show enhanced responsivity to exogenous OxA activation of the septo-hippocampal pathway, and (3) Aged animals undergo an intrinsic reduction in medial septum PV-immunoreactivity and a decrease in orexin innervation to remaining septal PV neurons. Alterations in orexin regulation of septo-hippocampal activity may contribute to age-related dysfunctions in arousal, learning, and memory.

Key words: aging, hippocampus, hypocretin, microdialysis, orexin, medial septum.

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Abbreviations: aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; CA1, cornu ammonis 1; GAD67, glutamic acid decarboxylase 67; LTP, long-term potentiation; OxA, orexin A; Ox1R, orexin 1 receptor; Ox2R, orexin 2 receptor; PV, parvalbumin; REM, rapid eye movement; RT, room temperature; SLM, stratum lacunosum moleculare; SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum.

The hippocampal formation is crucially involved in the execution of learning and memory processes. Alterations in hippocampal function and anatomy are likely contributors to age-related cognitive dysfunction (Rosenzweig and Barnes, 2003). In human patients, normal aging is associated with significant volumetric reductions in the hippocampal formation (Du et al., 2001), and rodent studies indicate population-specific neuronal loss in animal models of aging (Shetty and Turner, 1998; Vela et al., 2003; Stanley and Shetty, 2004; Gavilán et al., 2007; Stanley et al., in press).

While numerous studies have examined the effects of aging on intra-hippocampal function, alterations in extra-hippocampal input may also contribute to aging-induced hippocampal dysfunction. The septo-hippocampal pathway is comprised of cholinergic, GABAergic, and glutamatergic projections from the medial septum of the basal forebrain to the hippocampus (Frotscher and Léránth, 1985; Freund and Antal, 1988; Leranth and Frotscher, 1989; Sotty et al., 2003) and is instrumental to learning and memory as well as the maintenance of sleep–wake cycles (Lee et al., 1994; Bassant et al., 1995). Moreover, the medial septum is classically viewed as the hippocampal theta rhythm generator (Buzsáki, 2002). Theta activity is a 4–10 Hz rhythmic neuronal oscillation that occurs in the hippocampus during exploratory behavior and rapid eye movement (REM) sleep and is important for encoding of information by hippocampal place cells in learning and memory processes (Winson, 1978; Buzsáki, 2005).

Appropriately, lesion of the medial septum or septo-hippocampal fibers results in impaired performance on hippocampally mediated tasks (Becker et al., 1980; Hagan et al., 1988; Numan and Quaranta, 1990) and reductions in hippocampal theta power (Green and Arduini, 1954; Yoder and Pang, 2005). Many of the effects observed following disruption of the septo-hippocampal transduction are similar to age-related alterations in cognitive and homeostatic processes. For instance, alongside decreased performance on cognitive tasks, aging is also associated with alterations in rhythmic sleep–wake cycles and reductions in hippocampal theta power (Lamour et al., 1989; Abe and Toyosawa, 1999). Sleep cycles in the elderly people are characterized by an increase in fragmented daytime naps, earlier times of sleep onset, and alterations in the timing and frequency of nighttime REM and slow wave sleep (Miles and Dement, 1980; Bliwise, 1993; Dijk et al., 1999) compared to young counterparts.

Age-related alterations in homeostatic mechanisms, such as the disturbance in states of arousal and sleep, are

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suggestive of hypothalamic dysfunction. Indeed, numerous studies have indicated a significant reduction in the pro-arousal peptide orexin (hypocretin) over the aging spectrum (Porkka-Heiskanen et al., 2004; Zhang et al., 2005; Brownell and Conti, 2010; Kessler et al., 2011). Orexin cell bodies are localized to the perifornical lateral hypothalamus yet influence a vast number of homeostatic and physiological behaviors, such as feeding, attention, arousal, addiction, and cognition (de Lecea et al., 1998; Sakurai et al., 1998, 2010; Date et al., 1999; Jaeger et al., 2002; Telegdy and Adamik, 2002; Deadwyler et al., 2007; Aston-Jones et al., 2010), through widespread neuronal projections to regions that mediate these phenomena (Peyron et al., 1998; Date et al., 1999; Nambu et al., 1999).

Both cholinergic and GABAergic cells of the medial septum receive a dense afferent input from hypothalamic orexin projections (Eggermann et al., 2001; Wu et al., 2002, 2004), and orexin modulation of these projection cells is speculated to modulate arousal and hippocampal theta rhythm (España et al., 2001; Gerashchenko et al., 2001). While direct orexin projections to the hippocampus are not as robust as those to medial septum, previous studies support a significant role for orexin in performance on tasks of hippocampal-dependent cognition (Akbari et al., 2006, 2007, 2008) and the induction of long-term potentiation [LTP; (Selbach et al., 2004, 2010; Akbari et al., 2011)]. Orexin may control hippocampal neurotransmission through direct as well as transsynaptic modulation of various pathways, including the septo-hippocampal pathway, and these effects are largely undefined. Furthermore, age-related alterations in the orexin system may contribute to hippocampal dysfunction via both direct and transsynaptic mechanisms.

These studies were designed to examine the anatomical and neurochemical impact of aging on orexin modulation of hippocampal function, both directly, and by way of the medial septum, using immunohistochemistry and *in vivo* microdialysis. The hypothalamic neuropeptide orexin A (OxA) was infused into either CA1 or the medial septum while simultaneously measuring extracellular levels of hippocampal glutamate and GABA efflux in young and old rats. Moreover, orexin innervation of CA1 and the medial septum was assessed as a function of age in order to determine the impact of orexin modulation of hippocampal function over the course of normal aging.

EXPERIMENTAL PROCEDURES

Animals

All 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. Every effort was made to minimize the number of animals used and their suffering. Young (3–5 month) and aged (26–30 month) male, Fisher 344 Brown Norway F1 hybrid rats (National Institute of Aging breeding colony; Baltimore, MD, USA) were fed standard rat chow *ad libitum* and kept at a 12:12 light-dark cycle in a climate-controlled facility. All *in vivo* experiments were conducted during the light cycle.

In vivo microdialysis

Under sodium pentobarbital anesthesia (60–70 mg/kg), all rats received unilateral implantation of guide cannulae (Bioanalytical Systems, Inc. (BAS); West Lafayette, IN, USA) in the caudal hippocampus in the following coordinates relative to bregma: young—anterior −5.2 mm, lateral +3.8 mm at 10° angle, ventral −3.6 mm; aged—anterior −5.6 mm, lateral +4.0 mm at 10° angle, ventral −3.8 mm. A subset of rats received a second guide cannulae in the medial septum at the following coordinates relative to bregma: young—anterior +0.2 mm, lateral +1.0 mm at 8° angle, ventral −5.5 mm; aged—anterior +0.2 mm, lateral +1.0 mm at 8° angle, ventral −5.6 mm. After a 2-day recovery period following stereotaxic surgery, rats were habituated to the microdialysis bowls for three consecutive days before the onset of microdialysis sampling. On the morning of each dialysis session, stylets were removed and replaced with probes (BAS, 30 kDa cutoff) extending 2 mm beyond the ventral tip of guide cannulas. Probes were continuously perfused at 2 μl/min with artificial cerebrospinal fluid (aCSF; [in mM] NaCl 150, KCl 3.0, $CaCl_2$ 1.7, $MgCl_2$ 0.9, D-glucose 4.9, pH 6.9). Neostigmine (50 nM) was included in hippocampal aCSF (Fadel et al., 2005). Microdialysate collection began 3 h after probe insertion and consisted of 11 collections in 15-min intervals. During collections 5 through 8, the microdialysis inlet line in either the medial septum or hippocampus was switched to an aCSF solution containing either vehicle (aCSF), low orexin A (OxA; 0.1 μM; Bachem Americas, Inc.; Torrance, CA, USA; product No. H-4172) or high OxA (10 μM). Most animals were tested under all three conditions. Dialysates were stored at −80 °C until analysis for amino acids could be carried out by liquid chromatography. At the conclusion of dialysis sessions, animals were sacrificed, and brains were removed. Probe placement was assessed using an acetylcholinesterase background stain. Animals with probe tracts outside of the target region were excluded from results.

Microdialysis samples were analyzed by liquid chromatography with electrochemical detection (Fadel et al., 2005; Reznikov et al., 2007). After pre-column O-phthaldidialdehyde/beta-mercaptoethanol derivatization, glutamate and GABA were separated on a Unijet microbore 3 μm C18 column (BAS) using a mobile phase consisting of 0.1 M NaH_2PO and 28.5% methanol (pH 6.4) and detected at a glassy carbon electrode (+700 mV). Amino acid quantification was accomplished by comparison of peak areas with a daily three-point standard curve defining the expected range of glutamate and GABA values. Following quantification of glutamate and GABA, basal values of neurotransmitter release were defined by averaging values during pre-drug collections (microdialysis fractions 1–4). Values were then expressed as percent baseline to account for variability in basal efflux across sessions and between subjects. Microdialysis data were uncorrected for probe recovery.

Immunohistochemistry

All tissue was processed according to previously described protocol (Frederick-Duus et al., 2007; Reznikov et al., 2008). Briefly, a separate group of experimentally naive rats was deeply anesthetized using isoflurane and transcardially perfused with 0.1 M phosphate buffered saline and 4% paraformaldehyde. Whole brains were removed and post fixed overnight followed by cryoprotection in a 30% sucrose/0.1 M phosphate buffer solution. Tissue was coronally sectioned (45 μm thickness) on a cryostat using a 1:5 serial sectioning method (yielding five sets of tissue with adjacent sections 225 μm apart).

Free floating medial septum or hippocampal sections were incubated with a rabbit anti-OxA antibody (1:1000; Calbiochem;