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Expression of 5-HT₇ receptor mRNA in the hamster brain: effect of aging and association with calbindin-D28K expression

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Abstract

Aging affects several processes modulated by the 5-HT₇ receptor subtype, including circadian rhythms, learning and memory, and depression. Previously, we showed that aging induces a decrease in the hamster dorsal raphe (DRN) in both 5-HT₇ receptor binding and circadian phase resetting responses to 8-OH-DPAT microinjection. To elucidate the mechanisms underlying the aging decrease in 5-HT₇ receptors, we investigated aging modulation of 5-HT₇ receptor mRNA expression in the DRN, brain regions afferent to the DRN, and brain regions regulating circadian rhythms or memory. In situ hybridization for 5-HT₇ receptor mRNA was performed on coronal sections prepared from the brains of young, middle-aged, and old male Syrian hamsters. 5-HT₇ receptor mRNA expression was quantified by densitometry of X-ray film autoradiograms. The results showed that aging did not significantly affect 5-HT₇ receptor mRNA expression in the DRN or most other brain regions examined, with the exception of the cingulate cortex and paraventricular thalamic nucleus. Within the suprachiasmatic nucleus, the site of the master circadian pacemaker in mammals, 5-HT₇ receptor mRNA expression was localized in a discrete subregion resembling the calbindin subnucleus previously described. A second experiment using adjacent tissue sections showed that 5-HT₇ receptor mRNA and calbindin mRNAs were concentrated in the same region of the SCN, and as well as in the same region of several other brain structures. The localization of 5-HT₇ receptors and calbindin mRNAs within the same regions suggests that proteins they encode may interact to modulate processes such as circadian timekeeping.

Keywords

serotonin receptors; suprachiasmatic nucleus; hippocampus; raphe nuclei

1. Introduction

The 5-HT₇ receptor subtype, the most recently cloned of the serotonin receptor subtypes, modulates many physiological and behavioral functions, including circadian rhythms (Ehlen et al., 2001; Duncan et al., 2004; Duncan and Davis, 2005), rapid eye movement (REM) sleep (Hagan et al., 2000; Thomas et al., 2003; Monti and Jantos, 2006), body temperature (Hedlund et al., 2003), learning and memory (Roberts et al., 2004), and behavior (Guscott et al., 2005; Hedlund et al., 2005). For example, administration of the 5-HT₇ receptor-selective antagonists, SB-269970-A or DR-4004, to hamsters blocks phase advances of circadian locomotor activity rhythms induced by the serotonergic agonists, 8-OH-DPAT or 5-

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carboxamidotryptamine, demonstrating that pharmacological activation of 5-HT₇ receptors stimulates circadian phase resetting (Ehlen et al., 2001; Duncan et al., 2004). Also, administration of 5-HT₇ receptor-selective antagonists to guinea pigs or rats selectively decreases REM sleep, suggesting that endogenous serotonin acting at 5-HT₇ receptors exerts a tonic inhibition on REM sleep (Hagan et al., 2000; Thomas et al., 2003; Monti and Jantos, 2006). Furthermore, studies in mice have shown that deletion of the 5-HT₇ receptor gene impairs memory and learning (Roberts et al., 2004), reduces immobility in the Porsolt swim test, similar to antidepressants (Guscott et al., 2005; Hedlund et al., 2005), and inhibits hypothermia induced by 8-OH-DPAT (Hedlund et al., 2003).

Aging deleteriously affects some of the functions modulated by 5-HT₇ receptors, most notably, circadian rhythms and memory. Furthermore, some age-related changes in these processes have been associated with decreases in 5-HT₇ receptor expression. For example, significant attenuation of serotonergic induction of circadian phase shifts was observed in hamsters by 17–19 months of age (Penev et al., 1995; Duncan et al., 2004), the same age at which a significant reduction of specific 5-HT₇ receptor binding was exhibited in the dorsal raphe nucleus (DRN) (Duncan et al., 1999). Also, age-related memory deficits are exhibited by rodents and humans, and decreased expression of 5-HT₇ receptor mRNA in the ventral CA3 of the hippocampus has been observed in old rats (Kohen et al., 2000) [but see also (Yau et al., 1999)].

In order to further elucidate the mechanisms responsible for the age-related reduction of 5-HT₇ receptor binding in the aging hamster brain, we investigated 5-HT₇ receptor mRNA expression in young, middle-aged and old hamsters. We focused on the DRN, based on our previous identification of age-associated loss of 5-HT₇ receptor binding and functional responses in this region (Duncan et al., 1999; Duncan et al., 2004) and on brain regions known to send afferent projections to the DRN. We also investigated several other brain regions involved in the regulation of circadian rhythms, memory, or mood. In the course of investigating the expression of 5-HT₇ mRNA in the hamster suprachiasmatic nucleus, we observed that its expression was concentrated in a small subregion previously reported to express calbindin mRNA. Therefore, in this project, we also investigated the effect of aging on calbindin mRNA expression in the SCN and other brain regions.

2. Results

2.1. Experiment 1. The effect of aging on 5-HT₇ receptor mRNA expression in the dorsal raphe and other discrete regions of the midbrain and the forebrain

Expression of 5-HT₇ receptor mRNA was observed in many brain regions, similar to previous findings in rats (Neumaier et al., 2001). The DRN exhibited a relatively low level of 5-HT₇ receptor mRNA expression that was not significantly affected by aging (Fig. 1 and Table 1). Furthermore, no effect of aging on 5-HT₇ receptor mRNA expression was observed in most of the midbrain or forebrain regions examined, with the exception of the cingulate cortex and paraventricular thalamic nucleus (Fig. 1 and Table 1). Distinct but faint labeling was detected in a small circular region of the ventral mid-caudal SCN of young hamsters, but appeared to be absent from this region in most of the middle-aged and old animals.

2.2. Experiment 2. The effect of aging on 5-HT₇ receptor mRNA expression in the calbindin-expressing region of the SCN and on calbindin mRNA expression

Previous studies have indicated that the ventral mid-caudal region of the hamster SCN is unique in its expression of the calcium-binding protein calbindin, and furthermore, this region is essential for generation of circadian rhythms (LeSauter et al., 2002; Hamada et al., 2003; Kriegsfeld et al., 2004a; Antle and Silver, 2005). This experiment investigated if the

expression of 5-HT₇ receptor mRNA occurs in the same region of the SCN that expresses calbindin mRNA, and if aging effects the expression of 5-HT₇ receptor mRNA or calbindin mRNA within this region. The results showed that the ventral mid-caudal region of the hamster SCN expressed calbindin mRNA, as expected. Furthermore, 5-HT₇ receptor mRNA was co-expressed in the calbindin mRNA-expressing subnucleus (Fig. 2). The apparent age-related change in SCN 5-HT₇ receptor mRNA expression suggested in Experiment 1 was not confirmed in this study, as aging did not significantly affect 5-HT₇ receptor mRNA expression in the calbindin subnucleus of the SCN or calbindin mRNA expression itself (Fig. 2). (A third experiment investigating 5-HT₇ receptor mRNA expression in the SCN of a separate set of hamsters also did not detect significant age-related differences [data not shown].) In contrast to the SCN and DRN, significant age-related changes in calbindin mRNA expression were observed in the cingulate cortex, MRN, and LPAG (Table 2).

3. Discussion

Aging did not significantly alter 5-HT₇ receptor mRNA expression in the hamster DRN at ZT 6, and thus changes in transcription of this mRNA do not account for the age-associated decreases in 5-HT₇ receptor binding or functional responses previously observed in this brain structure (Duncan et al., 1999;Duncan et al., 2004). Furthermore, the relatively low levels of expression of 5-HT₇ receptor mRNA observed in the DRN (and MRN) suggest that some of the 5-HT₇ receptor protein identified in these regions by binding studies or functional studies may be located on afferent terminals of nerve fibers originating in other brain regions. Some of the brain regions that have been demonstrated to be afferent to the DRN in the rat, such as the bed nucleus of the stria terminalis, the medial preoptic nucleus, the anterior hypothalamic area, the ventral pontine periaqueductal gray, the cingulate cortex, and the paraventricular thalamic nucleus (Peyron et al., 1998;Gervasoni et al., 2000;Brown et al., 2002;Lee et al., 2005), exhibited 5-HT₇ receptor mRNA expression in the hamster brain, although other afferent regions, such as the lateral preoptic area, lateral hypothalamic area, lateral habenula, perifornical nucleus, posterior hypothalamic area, and medial tuberal nucleus, did not show 5-HT₇ receptor mRNA signal in this study. Of the DRN-afferent regions that expressed 5-HT₇ receptor mRNA, only two, the cingulate cortex and the paraventricular thalamic nucleus, exhibited age-related decreases in expression of this mRNA. If decreased 5-HT₇ receptor mRNA expression leads to decreased 5-HT₇ receptor protein on efferent projections from these regions to the DRN, then it could contribute to the age-related reduction in 5-HT₇ receptor mediated phase shifts that we have previously observed (Duncan et al., 2004). However, the lack of detectable age-related difference in 5-HT₇ receptor mRNA expression in most of the brain regions afferent to the DRN seems to suggest an alternative hypothesis, that age-related decrease in 5-HT₇ receptors in the DRN may be the result of some process other than decreased 5-HT₇ receptor mRNA expression, possibly neurotransmitter-induced down regulation. As shown in a previous study, aging is associated with a decrease in serotonin transporter binding in the hamster DRN (Duncan and Hensler, 2002), which would be likely to contribute to reduced serotonin reuptake and therefore increased extracellular serotonin concentrations that could induce 5-HT₇ receptor down regulation. This possibility is supported by findings that chronic treatment with serotonin selective reuptake inhibitors decreases 5-HT₇ receptor binding sites in rats (Mullins et al., 1999;Sleight et al., 1995).

As well as affecting circadian rhythms, the age-related decrease in 5-HT₇ receptor mRNA expression in the cingulate cortex may be related to age-associated changes in memory or affective state, because these processes that have been shown to be regulated by the cingulate cortex and modulated by 5-HT₇ receptors (Frankland et al., 2004;Maviel et al., 2004;Caetano et al., 2006;Mayberg, 2006;Roberts et al., 2004;Hedlund et al., 2005;Guscott et al., 2005). For example, the cingulate cortex participates in the regulation of contextual fear conditioning (Frankland et al., 2004) and in the affective response to pain (Johansen et al., 2001). Aging

leads to deficits in contextual fear conditioning (Gemma et al., 2004; Mesches et al., 2004; Gemma et al., 2006), as does mutation of the 5-HT₇ receptor (Roberts et al., 2004). If the age-related decrease in 5-HT₇ receptor mRNA expression in the cingulate cortex is also associated with a decrease in 5-HT₇ receptors, it would likely contribute to aging deficits in contextual fear conditioning.

In contrast to the cingulate cortex, the possible functional significance of age-related changes in 5-HT₇ receptor mRNA expression in the paraventricular thalamic nucleus is less clear. The processes that are regulated by the paraventricular thalamic nucleus, such as arousal and attention, as well as in the effects of stress on body temperature and energy balance (van der Werf et al., 2002; Kirouac et al., 2005; Bhatnagar and Dallman, 1999; Huang et al., 2006), have not been reported to be affected by 5-HT₇ receptor activation.

The lack of effect of aging on 5-HT₇ receptor mRNA expression in the hamster hippocampal ventral CA3 region in the current study contrasts with a previous report in rats, in which middle-aged and old animals exhibited significantly lower 5-HT₇ receptor mRNA expression than young animals (Kohen et al., 2000). The present results are consistent, however, with an earlier study in rats, which did not observe any age-associated changes in 5-HT₇ receptor mRNA expression in any hippocampal subfield (Yau et al., 1999).

The most interesting finding of the present study was the observation that 5-HT₇ receptor mRNA expression in the hamster SCN is concentrated in the calbindin subnucleus. This region is crucial for the generation of circadian rhythmicity, as shown by the loss of circadian rhythms in behavior (locomotor activity, drinking, gnawing) and circulating concentrations of hormones (melatonin and cortisol) in hamsters after discrete lesions of this area (LeSauter and Silver, 1999; Kriegsfeld et al., 2004b). Also, the calbindin subnucleus receives direct retinal innervation (Bryant et al., 2000) and mediates photic phase shifts, as demonstrated by the finding that intracerebroventricular administration of calbindin antisense oligonucleotides attenuates light induction of *Per1* mRNA expression in the SCN and circadian phase shifts (Hamada et al., 2003). The present identification of 5-HT₇ receptor mRNA within the calbindin subnucleus supports other findings that post-synaptic 5-HT₇ receptors in the SCN modulate the effects of serotonergic agonists on responses to light or to glutamate, the major neurotransmitter released by the retinohypothalamic tract that innervates the SCN (Ying and Rusak, 1997; Quintero and McMahon, 1999). Furthermore, the discrete localization of 5-HT₇ receptor mRNA within the calbindin mRNA expressing region of the SCN suggests that this receptor may modulate photic phase shifts through an interaction with calbindin.

The calbindin subnucleus is also innervated by serotonin- and neuropeptide Y- immunoreactive fibers (LeSauter et al., 2002; Antle and Silver, 2005). Because NPY projections from the IGL and serotonin projections from the median raphe are known to communicate nonphotic information to the suprachiasmatic nucleus (Meyer-Bernstein and Morin, 1998; Cutrera et al., 1994; Schuhler et al., 1999; Janik and Mrosovsky, 1994), it is possible that the calbindin subnucleus may be involved in mediating nonphotic resetting of the circadian pacemaker. One study investigating this possibility found that administration of calbindin antisense oligonucleotides did not affect phase shifts induced by injections of the 5-HT_{1A/7/5} agonist, 8-OH-DPAT, although a small sample size (N=3) was used (Hamada et al., 2003). However, it should be noted that photic phase shifts involve an increase in *Per1* mRNA expression in the SCN, especially in the calbindin subregion, and that this increase is attenuated by administration of calbindin antisense oligonucleotides (Hamada et al., 2003). Because induction of phase shifts by 8-OH-DPAT injection or other nonphotic signals is associated with decreased *Per1* mRNA expression (Horikawa et al., 2000; Duncan et al., 2005; Fukuhara et al., 2001; Hamada et al., 2004), it is not surprising that calbindin antisense oligonucleotide

attenuation of SCN *Per1* expression does not prevent phase shifts to 8-OH-DPAT (Hamada et al., 2003).

In the present study, neither calbindin mRNA expression in the SCN nor 5-HT₇ receptor mRNA expression in the calbindin subnucleus of the SCN were significantly affected by aging. Thus, the age-related attenuation of photic induction of phase shifts and SCN *Per* mRNA expression that has been previously reported in hamsters (Kolker et al., 2003; Zhang et al., 1996) apparently is not mediated by decreased calbindin mRNA expression in the SCN. Furthermore, the present findings in the hamster brain are consistent with previous studies in rats indicating that age-associated changes in calbindin mRNA occur only in discrete regions. In aging rats, decreases in calbindin mRNA or protein expression were observed only in the cerebellum, corpus striatum and nucleus basalis and not in most brain regions examined (Iacopino and Christakos, 1990). Age-related decreases in calbindin mRNA expression in the hamster brain were observed only in the cingulate cortex, the median raphe nucleus, and the lateral periaqueductal gray. The significance of these age-related changes is not known, because the function of calbindin in these brain regions has not been elucidated.

The findings presented here show that 5-HT₇ receptor mRNA is expressed in regions of the SCN and other brain structures where calbindin mRNA is expressed. Because a specific antibody that distinctly labels 5-HT₇ receptors in hamster brain tissue is not available, we have been unable to investigate whether 5-HT₇ receptor protein is co-localized at the cellular level with calbindin protein. It is interesting to note that immunohistochemical studies have shown that another serotonin receptor subtype, the 5-HT_{1A} receptor, is co-localized with calbindin in several rat brain regions, including the hippocampus, the thalamus and the septum (Aznar et al., 2003). As well as modulating photic phase shifts (Hamada et al., 2003), as described above, calbindin has been implicated in several other functions, including spatial memory, long-term potentiation in the hippocampus, synaptic plasticity, and either neuroprotection or enhanced vulnerability to neurodegeneration (Jouvenceau et al., 1999; Chard et al., 1995; Molinari et al., 1996; Nagerl et al., 2000). Some of these functions, such as memory, long-term potentiation, and synaptic plasticity are also modulated by 5-HT₇ receptors (Perez-Garcia et al., 2006; Perez-Garcia and Meneses, 2005; Roberts et al., 2004; Kvachnina et al., 2005).

4. Conclusions

5-HT₇ receptor mRNA expression in the DRN and most other hamster brain regions examined is not significantly affected by aging. Within the SCN, 5-HT₇ receptor mRNA expression is concentrated in the subregion that expresses calbindin mRNA. Expression of calbindin mRNA was also observed in several other brain regions that express 5-HT₇ receptor mRNA, including the cingulate cortex, median raphe nucleus, and hippocampal CA1 and CA2 subfields.

Localization of mRNAs for both the 5-HT₇ receptor and calbindin within the same regions suggests the potential for interaction of these two proteins for modulation of processes such as circadian rhythms and memory. The present findings show that aging affects expression of 5-HT₇ mRNA and calbindin mRNA differentially and in a brain region specific manner. If decreases in mRNA expression predict decreases in protein expression, then these decreases may contribute to age-related decrements in the functions subserved by these proteins in discrete brain regions.

5. Experimental procedures

5.1. Animals and tissue preparation

Male Syrian hamsters (Harlan HSD) of three ages (young, 3–5 months; middle-aged, 12–14 months; old, 17–19 months; N=6–9/age/experiment) were used. After exposure to a light:dark

cycle (lights on from 0600–2000 h) for at least ten days, the hamsters were decapitated at 1400 h (i.e., zeitgeber time [ZT] 6, where ZT 12 is conventionally considered the time of lights off), the time at which serotonergic drugs induce maximal circadian phase advances (Tominaga et al., 1992). After dissection, brains were frozen on crushed dry ice and stored at -80°C . Coronal sections (20 μm thick) through the hypothalamus and midbrain were cut with a cryostat and mounted onto negatively charged slides. The experimental procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee prior to implementation.

5.2. In situ hybridization

Partial cDNA sequences of hamster 5-HT₇ receptor (nt 1–765) and calbindin-D28K (nt 179–658) were synthesized by RT-PCR and cloned into pBluescript SK(+). Plasmid DNA was purified, linearized, and subjected to c-RNA synthesis using ³⁵S-UTP as the label. Four tissue sections per animal per anatomical region were used for each in situ hybridization experiment. Slide-mounted tissue sections were equilibrated to room temperature, fixed for 15 min in 4% PFA/0.1M PB (pH 7.4) and washed with PBS. The sections were acetylated in 0.1 M triethanolamine/0.25% acetic anhydride, dehydrated and delipidated, and air dried.

The sections were hybridized for 20 h at 55°C with riboprobes diluted in hybridization cocktail (250 mg/ml salmon sperm DNA, 625 mg/ml yeast total RNA, 20 mM Tris-HCl, 1 mM EDTA, 300 mM NaCl, 50% v/v deionized formamide, 10% v/v dextran sulphate, 1X Denhardt's solution, 100 mM dithiothreitol, 0.1% SDS, and 0.1% Na-thiosulfate). After hybridization, the sections were rinsed in 2X SSC/10 mM DTT at 22°C , treated with RNase (40 mg/ml) at 37°C , washed in 1X SSC and incubated for 1 h at 63°C in 0.1X SSC. Then the sections were washed (0.1X SSC, 22°C), dehydrated and air dried. Experimental slides and ¹⁴C-standards (Amersham Biosciences) were apposed to X-ray film (Kodak Biomax-MR) for 2–4 weeks. The X-ray films were developed using standard procedures. The resulting autoradiograms were analyzed using computer-assisted microdensitometry (MCID, Imaging Research Inc.), as described previously (Duncan et al., 2001).

5.3. Experiment 1. The effect of aging on 5-HT₇ mRNA expression in the dorsal raphe and other discrete regions of the midbrain and the forebrain

This study investigated whether aging is associated with changes in 5-HT₇ receptor mRNA expression in the DRN, and other circadian substrates, e.g., the suprachiasmatic nucleus (SCN), the intergeniculate leaflet (IGL), and the median raphe (MRN). Additionally, brain regions afferent to the DRN were measured, including the cingulate cortex, bed nucleus of the stria terminalis (BNST), lateral septum, central nucleus of the amygdala, anterior hypothalamus, anterior paraventricular thalamic nucleus, zona incerta, and lateral periaqueductal gray (LPAG) (Gervasoni et al., 2000; Rampon et al., 1999). Finally, the dentate gyrus (DG) and fields CA1, CA2, and CA3 of the hippocampus were also studied. The brain sections used corresponded to plates 18–20 (lateral septum, BNST, and cingulate cortex), 24–25 (SCN, central nucleus of the amygdala, anterior hypothalamus, and anterior paraventricular thalamic nucleus), 29–31 (IGL and zona incerta), plates 33–35 (hippocampus) or plates 41–42 (DRN, MRN, and LPAG) shown in *A Stereotaxic Atlas of The Golden Hamster Brain*, by L.P. Morin and R.I. Wood (Academic Press, 2001).

5.4. Experiment 2. The effect of aging on 5-HT₇ mRNA expression in the calbindin-expressing region of the suprachiasmatic nucleus

The results of Experiment 1 indicated that expression of 5-HT₇ receptor mRNA is localized to a small subregion of the SCN that resembled the calbindin subnucleus previously described in the hamster SCN (LeSauter et al., 2002; Hamada et al., 2003; Kriegsfeld et al., 2004a; Antle and Silver, 2005). Therefore, this experiment investigated if the expression of 5-HT₇ receptor mRNA occurs in the same region of the SCN that expresses calbindin mRNA, and if aging

effects the expression of 5-HT₇ receptor mRNA or calbindin mRNA within this region. Adjacent sections of the mid-caudal SCN were used for in situ hybridization for these two mRNA species. For each animal, the boundary of the autoradiogram representing calbindin mRNA expression was traced and superimposed over the autoradiogram representing 5-HT₇ receptor mRNA expression in an adjacent SCN section, and 5-HT₇ receptor mRNA expression within this region was determined.

5.5 Data and statistical analyses

The data for each brain region were subjected to one-way ANOVA, assessing the effect of age. In the case of significant P values (P<0.05), Bonferroni's test was conducted.

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Abbreviations

Cg	cingulate cortex
LS/LSV	lateral septum (ventral)
TS	triangular septal nucleus
BNST	bed nucleus of the stria terminalis
CPu	

	caudate putamen
SCN	suprachiasmatic nucleus
AH	anterior hypothalamus
Ce	central amygdaloid nucleus
MHb	median habenula
PVA	anterior paraventricular thalamic nucleus
ZI	zona incerta
PMV	ventral premammillary nucleus
IGL	intergeniculate leaflet
DG	dentate gyrus
CA1	CA1 region of the hippocampus
CA2	CA2 region of the hippocampus
CA3	CA3 region of the hippocampus
MRN	median raphe nucleus
DRN	dorsal raphe nucleus
LPAG	lateral periaqueductal gray

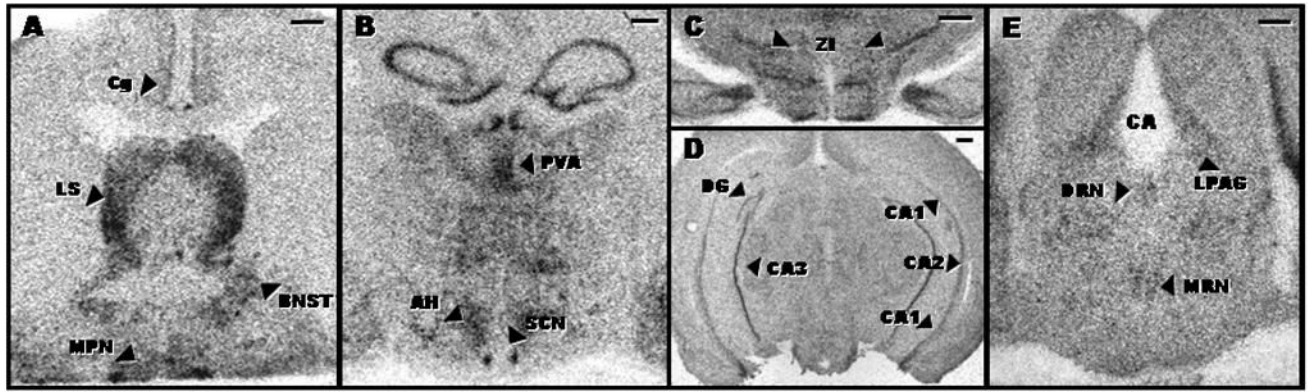


Figure 1.
Autoradiograms representing expression of 5-HT₇ receptor mRNA in the forebrain and midbrain of young adult hamsters. Scale bar depicts 500 microns.

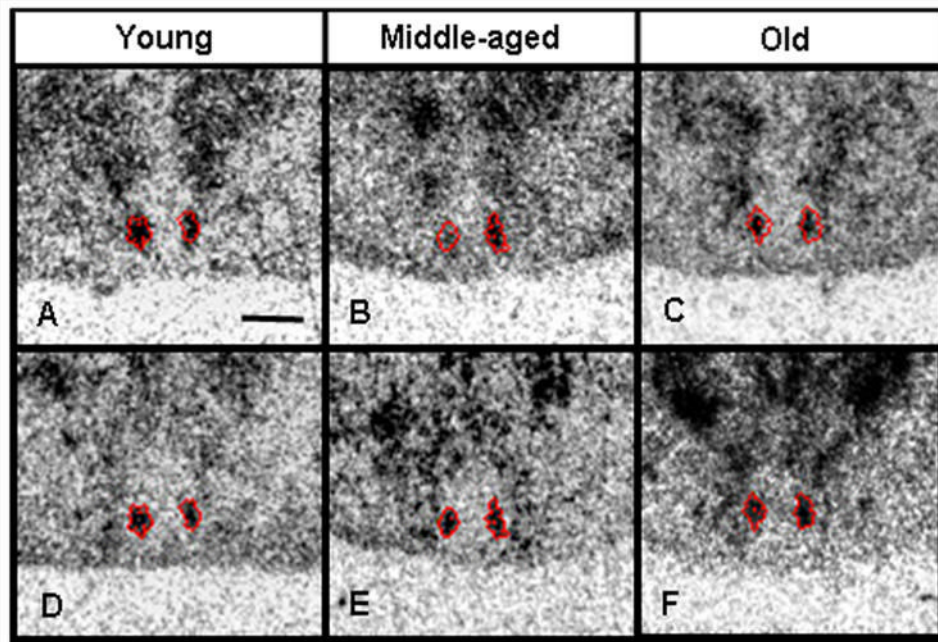


Figure 2. Autoradiograms demonstrating expression of 5-HT₇ receptor mRNA and calbindin mRNA in the same region of the hamster SCN. The red traces represent the border of the calbindin mRNA expression. Scale bar depicts 500 microns.

Table 15-HT₇ receptor mRNA expression in brain regions of young, middle-aged, and old hamsters.

Brain Region	Young		Middle-aged		Old		P
	Mean	SEM	Mean	SEM	Mean	SEM	
Cortex	8.6*	1.1	8.7	1.2	5.4	0.6	0.04
Cg	6.53	1.8	7.4	1.0	8.4	1.0	0.58
DG	39.0	6.1	45.0	9.3	27.0	4.1	0.19
CA1	50.8	17.8	50.2	8.8	40.8	3.2	0.77
CA2	76.8	4.8	74.1	4.3	78.9	4.3	0.74
CA3							
Basal forebrain	20.0	2.0	17.3	3.0	11.6	2.0	0.06
LS	12.0	1.0	12.5	1.7	10.3	0.9	0.44
BNST							
Hypothalamus	76.4	13.6	72.9	29.0	108.0	16.8	0.44
SCN	61.9	4.0	56.3	5.9	54.4	3.2	0.48
AH							
Amygdala	87.7	7.5	93.4	12.0	75.6	10.3	0.46
Ce							
Thalamus	16.2	2.6	15.2	1.7	12.2	1.0	0.30
MHb	384.0	27.9	322.0	22.6	295.0	18.3	0.03
PVA	128.0	3.4	114.0	5.9	119.0	2.7	0.07
ZI	41.3	4.5	32.1	5.2	39.9	6.7	0.49
IGL							
Mid-brain	10.4	1.3	12.0	2.4	14.5	2.2	0.34
MRN	11.1	1.6	13.4	2.4	11.0	2.2	0.70
DRN	17.7	2.0	22.2	1.8	18.7	1.7	0.22
LPAG							

* Values represent the mean of 6–9 animals.

AH, anterior hypothalamus; BNST, bed nucleus of the stria terminalis; CA1, CA1 region of the hippocampus; CA2, CA2 region of the hippocampus; CA3, CA3 region of the hippocampus; Ce, central nucleus of the amygdala; Cg, cingulate cortex; DG, dentate gyrus; DRN, dorsal raphe nucleus; IGL, intergeniculate leaflet; LPAG, lateral periaqueductal gray; LS, lateral septum; MHb, medial habenula; MRN, median raphe nucleus; PVA, anterior paraventricular thalamic nucleus; SCN, suprachiasmatic nucleus; ZI, zona incerta

Table 2
Calbindin D28K mRNA expressions in brain regions of young, middle-aged, and old hamsters.

Brain Region	Young		Middle-aged		Old		P
	Mean	SEM	Mean	SEM	Mean	SEM	
Cortex	81.9*	6.9	53.5	4.3	51.7	5.5	0.002
Cg	207.0	12.0	166.0	15.1	179.0	15.1	0.15
CA1	128.0	9.5	97.2	11.6	101.0	15.7	0.22
CA2							
Basal forebrain	87.1	9.8	68.0	8.8	84.6	7.2	0.27
LS ventral	135.0	40.4	166.0	42.3	193.0	29.6	0.57
TS	66.8	12.7	93.8	18.0	73.2	9.9	0.37
BNST							
Basal ganglia	58.2	4.5	48.3	5.8	53.0	4.8	0.42
CPu							
Hypothalamus	84.5	9.0	83.3	8.9	60.0	5.0	0.10
SCN	299.0	65.8	266.0	44.9	287.0	39.4	0.90
PMV							
Mid-brain	38.1	10.0	10.3	5.2	12.0	3.4	0.02
MRN	162.0	25.7	144.0	29.1	141.0	21.1	0.80
DRN	84.8	8.6	56.0	9.8	53.3	6.9	0.03
LPAG							

* Values represent the mean of 6–9 animals.

BNST, bed nucleus of the stria terminalis; CA1, CA1 region of the hippocampus; CA2, CA2 region of the hippocampus; Cg, cingulate cortex; CPu, caudate putamen; DRN, dorsal raphe nucleus; LPAG, lateral periaqueductal gray; LS ventral, ventral lateral septum; MRN, median raphe nucleus; PMV, ventral preammillary nucleus; SCN, suprachiasmatic nucleus; TS, triangular septal nucleus