

MINI-REVIEW

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Influence of age on nicotinic cholinergic regulation of blood flow in rat's olfactory bulb and neocortex

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Abstract

The olfactory bulb receives cholinergic basal forebrain inputs as does the neocortex. With a focus on nicotinic acetylcholine receptors (nAChRs), this review article provides an overview and discussion of the following findings: (1) the nAChRs-mediated regulation of regional blood flow in the neocortex and olfactory bulb, (2) the nAChR subtypes that mediate their responses, and (3) their activity in old rats. The activation of the $\alpha 4\beta 2$ -like subtype of nAChRs produces vasodilation in the neocortex, and potentiates olfactory bulb vasodilation induced by olfactory stimulation. The nAChR activity producing neocortical vasodilation was similarly maintained in 2-year-old rats as in adult rats, but was clearly reduced in 3-year-old rats. In contrast, nAChR activity in the olfactory bulb was reduced already in 2-year-old rats. Thus, age-related impairment of $\alpha 4\beta 2$ -like nAChR function may occur earlier in the olfactory bulb than in the neocortex. Given the findings, the vasodilation induced by $\alpha 4\beta 2$ -like nAChR activation may be beneficial for neuroprotection in the neocortex and the olfactory bulb.

Keywords Olfactory bulb, Neocortex, Cerebral blood flow, Nicotinic acetylcholine receptor, Rat

Introduction

As background information for this review article, a brief history and scientific knowledge of basic and clinical research on the basal forebrain cholinergic system will be provided. In humans, the olfactory function starts declining after 65 years of age [1]. In addition to age-related impairment, olfactory decline is an early symptom of Alzheimer's disease (AD) that appears before cognitive decline [2, 3]. The olfactory bulb, which is the first processing station of olfactory information in the brain, receives cholinergic basal forebrain input, as do the neocortex and hippocampus, which contribute to

cognition and memory, respectively [4]. More specifically, magnocellular neurons in the basal forebrain provide widespread cholinergic innervation to the neocortex, hippocampus, and olfactory bulb. The bilateral horizontal and caudal portions of the cholinergic basal forebrain neurons, which are located in the substantia innominata (SI) and nucleus basalis of Meynert (NBM), project their axons to the neocortex. In turn, fibers of neurons located in the nucleus of the horizontal limb of the diagonal band of Broca (HDB) project to the olfactory bulb. The most rostral level of the cholinergic neurons of the basal forebrain, which is located in the medial septal nucleus (MS) and the nucleus of the vertical limb of the diagonal band of Broca (VDB), project mainly to the hippocampus [4, 5]. These cholinergic neurons of the basal forebrain undergo selective degeneration in patients with AD [6, 7]. Moreover, individual variations in cholinergic cell loss, from moderate to severe, are correlated with the degree of cognitive deterioration in these patients [7].

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This indicates the importance of this cholinergic system for cognitive function. In vivo brain imaging studies have revealed a moderate structural decline in the gray matter volume of the basal forebrain cholinergic system during the adult life span, which worsens with advanced age [8]. A further decrease in the volume of the basal forebrain cholinergic system beyond the age effect alone were detected in early stages of AD [8, 9].

Presumed cholinergic terminations onto cerebral blood vessels from the magnocellular basal nucleus, in addition to interneuronal contacts, are evident in rodent studies that used the anterograde axonal-tracing technique [10]. The activation of cholinergic fibers originating in the NBM produces vasodilation, which leads to increases in regional blood flow in the neocortex in anesthetized rats [11–13]. Acetylcholine receptors consist of muscarinic and nicotinic receptors. The activation of not only muscarinic but also nicotinic receptors within the parenchyma of the neocortex is involved in the NBM cholinergic vasodilative system [11].

In the human neocortex, nicotinic acetylcholine receptors (nAChRs) exhibit a greater decline than do muscarinic acetylcholine receptors during the normal aging process as well as in patients with AD [14, 15]. Therefore, to understand the mechanisms of olfactory decline associated with cognitive decline in older adults and patients with AD, it is important to elucidate the regulation of regional blood flow mediated by the nicotinic cholinergic system in the neocortex and olfactory bulb, together with its aging process.

In this article, we focus on nAChRs, review results reported mainly by our research group using anesthetized rats and discuss on (1) nAChRs-mediated regulation of regional blood flow in the neocortex and the olfactory bulb, (2) nAChR subtype mediating their responses, and (3) their activity in old rats.

Nicotinic cholinergic regulation of regional blood flow in the neocortex

Nicotine injection

Nicotine, a nAChR agonist, has been demonstrated to increase regional cerebral blood flow when injected intravenously, especially in the neocortex, independent of mean arterial pressure [16–18]. In our investigation, we measured the blood flow in the frontal cortex by laser Doppler flowmetry in urethane-anesthetized artificially ventilated rats, before and after intravenous bolus injection of nicotine [18]. Nicotine at doses of 3–30 µg/kg increased neocortical blood flow in a dose-dependent manner, without significant changes in mean arterial pressure. At 300 µg/kg, nicotine increased neocortical blood flow in parallel with a marked increase in arterial pressure. The rapid increase in neocortical blood flow

after the injection of 300 µg/kg of nicotine appears to be due to a passive increase in neocortical blood flow in response to the increased arterial pressure. Accordingly, nicotine doses of 30 µg/kg or less increased the neocortical blood flow without affecting systemic blood pressure is due to active dilation of neocortical vessels. Nicotine at the same doses had a similar effect to the parietal cortex in our animals.

The finding mentioned above is in line with evidence that the activation of nAChRs is involved in vasodilation in the neocortex by excitation of intracranial cholinergic fibers originating in the NBM of the basal forebrain projecting to the neocortex [11–13, 19].

nAChR subtype

The increase in neocortical blood flow induced by intravenous injection of nicotine (30 µg/kg) is due to an activation of nAChRs in the brain; in fact, the response was not influenced by a nAChR antagonist (hexamethonium) which cannot transverse the blood–brain barrier, but was abolished by a nAChR antagonist (mecamylamine) that can cross it [18]. Activations of nAChRs in both the NBM and the neocortex are possibly involved in the nicotine-induced neocortical vasodilation [17, 18]. Furthermore, nitric oxide is necessary for this nicotine-induced increase in neocortical blood flow [20].

Of the various subtypes of nAChRs, the $\alpha 4\beta 2$ and $\alpha 7$ subtypes are the most abundant and widespread in the mammalian brain, including the neocortex and NBM [21–23]. The increase in neocortical blood flow induced by nicotine was not influenced by methyllycaconitine, an $\alpha 7$ -selective nAChR antagonist but was completely abolished by dihydro- β -erythroidine, an $\alpha 4\beta 2$ -preferring nAChR antagonist [24]. These results suggest that activation of $\alpha 4\beta 2$ -like nAChRs but not of $\alpha 7$ nAChRs in the NBM and the neocortex is responsible for the nicotine-induced neocortical vasodilation. However, although dihydro- β -erythroidine is often used as an $\alpha 4\beta 2$ -preferring nAChR antagonist [25, 26], we cannot exclude the possible contribution of other heterometric nAChRs, since dihydro- β -erythroidine can bind to heterometric neuronal nAChRs containing not only the $\alpha 4\beta 2$ subtype but also the $\alpha 4\beta 4$ [27], $\alpha 3\beta 2$ [28], and $\alpha 2\beta 2$ [29] subtypes.

Aging

The above-mentioned experiments showing an increase in neocortical blood flow induced by intravenous injection of nicotine (30 µg/kg) were performed in adult rats aged 3–10 months. Then we moved on to older animals [18, 30, 31]. In rats 23–26 months old (approximately 2 years old), a bolus injection of 30 µg/kg of nicotine increased neocortical blood flow to a similar

extent as in the younger rats. A lower nicotine dose (3 $\mu\text{g}/\text{kg}$), however, was ineffective. In other words, the intensity of the response to nicotine remained unchanged in old rats, but the threshold became higher. In contrast, in 32–36 months old rats (approximately 3 years old), nicotine, at 3 or 30 $\mu\text{g}/\text{kg}$, had no significant effect on the neocortical blood flow (Fig. 1A). The decrease in the neocortical blood flow response in the very old animals is probably due to a decline in the number of nAChRs in the neocortex, as previously observed in both rodents and humans [14, 32].

Nicotinic cholinergic regulation of regional blood flow in the olfactory bulb

Nicotine injection

The effect of an intravenous bolus injection of nicotine on the blood flow in the olfactory bulb was investigated [33]. Nicotine at a dose of 30 $\mu\text{g}/\text{kg}$, increased neocortical blood flow [18], but did not increase blood flow in the olfactory bulb [33]. This result agrees with the fact that activation of the HDB in the basal forebrain, which is the main source of cholinergic input to the olfactory bulb [5, 34, 35], increased extracellular acetylcholine release in the olfactory bulb but failed to affect the blood flow in

the olfactory bulb [36]. Our results suggest a functional difference between the olfactory bulb and neocortex regarding cerebral blood flow regulation through cholinergic activation.

Multiple *in vivo* studies in rodents have described that natural olfactory stimulation increases regional blood flow in the olfactory bulb, in association with neuronal activities [37–40]. The vasodilation in the olfactory bulb induced by olfactory stimulation is due to neurovascular coupling mechanisms [41, 42].

Next, we investigated the effect of nAChR activation by nicotine injection on the blood flow response in the olfactory bulb induced by olfactory stimulation in urethane-anesthetized artificially ventilated rats [43]. Odor stimulation (5% amyl acetate, 30 s) produced an increase in olfactory bulb blood flow without changes in frontal cortical blood flow or mean arterial pressure. An intravenous injection of nicotine at a dose of 30 $\mu\text{g}/\text{kg}$ potentiated the odor-induced increased olfactory bulb blood flow, without changing the basal blood flow level.

The olfactory nerve transmits smell information from the olfactory epithelium to the olfactory bulb. Rodent studies indicated that the olfactory nerve increases its firing frequency depending on the odor concentration

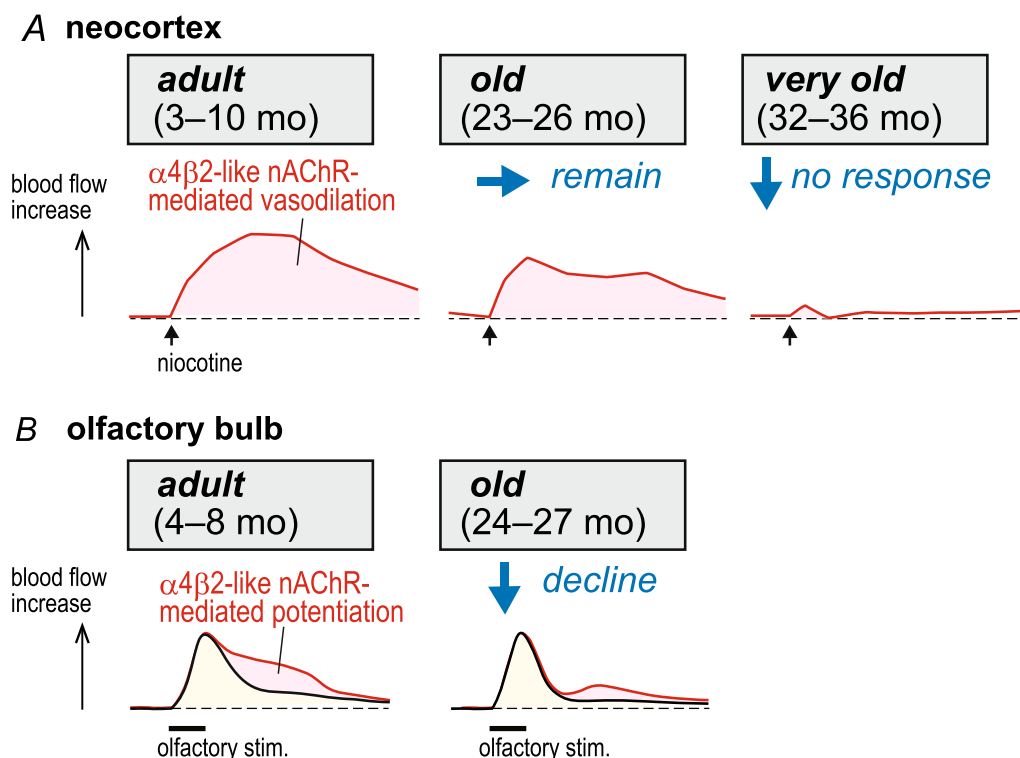


Fig. 1 Schematic diagram showing the aging effects of $\alpha4\beta2$ -like nAChR-mediated neocortical vasodilation (**A**) and potentiation of vasodilation in the olfactory bulb (**B**). **A** Neocortical vasodilation induced by intravenous bolus injection of nicotine at a dose of 30 $\mu\text{g}/\text{kg}$ was schematically illustrated [18]. **B** Vasodilation of the olfactory bulb induced by olfactory nerve stimulation before (black line) and after (red line) intravenous injection of nicotine at a dose of 30 $\mu\text{g}/\text{kg}$ was schematically illustrated [54]. nAChR nicotinic acetylcholine receptor

[44–46]. By varying the stimulus frequencies of olfactory nerve electrical stimulation, we could quantify the strength of odor stimulation. Our experiments demonstrated that unilateral, electrical stimulation of olfactory nerve produced current ($\geq 100 \mu\text{A}$) and frequency-dependent ($\geq 5 \text{ Hz}$) increases in blood flow in the olfactory bulb ipsilateral to the stimulus without changes in frontal cortical blood flow or mean arterial pressure [47]. Furthermore, we observed that an intravenous injection of nicotine ($30 \mu\text{g/kg}$) augmented the olfactory bulb blood flow response to nerve stimulation. Nicotine-induced potentiation of olfactory bulb blood flow responses occurred with olfactory nerve stimulation at 2 and 20 Hz but not at 100 Hz [47]. This finding provides additional evidence that nAChR activation potentiates olfactory bulb blood flow responses to olfactory input, this potentiation occurs for intermediate or weak, but not strong, input. In a Ca^{2+} imaging study in the mouse olfactory bulb, Bendahmane et al. [48] indicated that electrical stimulation of the HDB leads to the activity-dependent modulation of glomerular odor responses, whereby weak-to-moderate responses are enhanced and strong responses are reduced. Thus, our results [47], almost agree with those of Bendahmane et al. [48].

nAChR subtype

The above-mentioned nicotine-induced potentiation of olfactory bulb blood flow response to odor was negated by dihydro- β -erythroidine, an $\alpha 4\beta 2$ -preferring nAChR antagonist [43]. Thus, our results suggest that the activation of $\alpha 4\beta 2$ -like neuronal nAChRs in the brain potentiates olfactory sensory processing in the olfactory bulb. However, a contribution of not only $\alpha 4\beta 2$ -like nAChRs [49] but also $\alpha 2$ - [50] and $\beta 4$ -containing nAChRs [51] in the olfactory bulb should be considered, since dihydro- β -erythroidine can bind to heterometric neuronal nAChRs other than the $\alpha 4\beta 2$ subtype.

As described in “nAChR subtype” section, activation of $\alpha 4\beta 2$ -like nAChRs in the NBM and the neocortex is suggested to be responsible for the nicotine-induced neocortical vasodilation [18, 24]. The olfactory bulb receives cholinergic neural inputs originating in the HDB in the basal forebrain [5, 35]. Both in olfactory bulb and HDB cholinergic neurons, mRNA expression of both the $\alpha 4$ and $\beta 2$ nAChR subunits has been identified in rats [21, 52, 53]. Thus, the nicotine-induced potentiation of olfactory sensory processing in the olfactory bulb could be due to activation of $\alpha 4\beta 2$ -like neuronal nAChRs in the olfactory bulb and/or in HDB cholinergic neurons.

Aging

The investigation of nicotine-induced potentiation of olfactory bulb blood flow responses induced by olfactory nerve stimulation in adult rats (4–8 months old) described above was then extended to animals of older age [54]. In old rats of 24–27 months (approximately 2 years old), olfactory nerve stimulation produces vasodilation in the olfactory bulb. However, the nicotine-induced potentiation of olfactory bulb vasodilation due to $\alpha 4\beta 2$ -like nAChR activation decreased considerably in old rats (Fig. 1B). In contrast, the olfactory bulb vasodilatory response to hypercapnic stimulation, indicating the vasodilatory ability of the olfactory bulb, was considerably greater than its response to olfactory nerve stimulation. Thus, we consider that with age, the olfactory bulb blood vessels maintain their vasodilatory ability but with lower reactivity to nicotine. This suggests a decline in $\alpha 4\beta 2$ -like nAChR function involving the nicotine-induced potentiation of olfactory bulb vasodilation in old rats.

In old rats of 24–27 months, electrical stimulation of unilateral olfactory nerve increased blood flow in the olfactory bulb ipsilateral to the stimulus without changes in mean arterial pressure [54]. The spatiotemporal blood flow response characteristics and the current and frequency dependence of prompt vasodilation of the olfactory bulb were identical to those observed in adult rats [47]. This is consistent with Kass et al. [55], who described that the odor-evoked synaptic output from the olfactory sensory neurons to the olfactory bulb glomeruli is relatively stable in anesthetized mice of 6–24 months old.

Comparison of aging effects on the blood flow responses in the neocortex and the olfactory bulb

As described above, the $\alpha 4\beta 2$ -like nAChR-mediated vasodilation in the neocortex induced by nicotine injection is relatively well maintained in old rats (23–26 months old) but markedly declines in very old rats (32–36 months old) [18, 24]. On the other hand, the $\alpha 4\beta 2$ -like nAChR-mediated potentiation of olfactory bulb vasodilation induced by nicotine injection is reduced in old rats (24–27 months old) [54]. Accordingly, we assume that the age-related impairment of $\alpha 4\beta 2$ -like nAChR function may affect the olfactory bulb earlier than the neocortex.

In rat brains, $\alpha 4$ and $\beta 2$ mRNA levels decrease from 7 to 29 months of age, further decreasing at 32 months. This tendency is relatively constant across in different areas of the brain including neocortex, although olfactory bulb is not analyzed [56]. Similarly, in human neocortex, decreases in $\alpha 4$ and $\beta 2$ mRNA levels as well as $\alpha 4\beta 2$

nAChR availability have been reported with normal aging [57, 58] and AD [59]. The diminished olfactory bulb blood flow potentiation effects of nicotine in old rats as well as diminished nicotine-induced neocortical vasodilation in very old rats may be due to the decline in $\alpha 4\beta 2$ nAChRs in the brain. Further studies comparing the aging effects on neocortex and olfactory bulb, regarding $\alpha 4\beta 2$ nAChR availability, are needed.

Age-related impairment of the regulation of blood flow in the olfactory bulb and neocortex mediated by $\alpha 4\beta 2$ -like nAChRs may not be comparable to the baseline regional cerebral blood flow, at least in rodents. This is because the baseline regional blood flow in the neocortex and olfactory bulb is not significantly different in 12-, 24-, and 34-month-old conscious rats when measured using the [^{14}C]-iodoantipyrine method [60]. Similarly, using microsphere methods, the baseline regional blood flow in the olfactory bulb has been shown to remain unchanged in 6- and 24-month-old conscious rats [61]. In contrast, in the human brain, resting (baseline) gray matter cerebral blood flow, including that in the frontal regions, is decreased between the ages of 40 and 100 years, as measured using the ^{133}X inhalation method [62]. Similar age-related reductions in gray matter cerebral blood flow have been reported by studies that used positron emission tomography [63] and pseudocontinuous arterial spin labeling (pCASL) MRI [64].

Clinical significance

This review emphasized the crucial role of $\alpha 4\beta 2$ -like nAChRs in the brain in neocortical vasodilation and potentiation of olfactory bulb vasodilation in response to olfactory stimulation. A considerable decrease of these $\alpha 4\beta 2$ -like nAChR functions occur in older animals at around 2 years old in the olfactory bulb, and later at around 3 years old in the neocortex. The age-related impairment of nicotinic cholinergic regulation of cerebral blood flow in the neocortex and olfactory bulb may explain the deterioration of olfactory and cognitive function in older people [65, 66]. The earlier decline of nAChR function in the olfactory bulb than in the neocortex may explain why the olfactory dysfunction is the earliest symptoms of AD [2, 3]. Human studies have described the relationship between olfaction, cognitive function, regional cerebral blood flow, and the nicotinic cholinergic system. Pilot studies among community-dwelling older adults have shown that older individuals with a higher olfactory identification threshold for rose odor exhibited a greater decline in cognitive function, particularly in attention and discrimination abilities [67, 68]. Attention and discrimination abilities are related to the basal forebrain cholinergic system [69, 70] and undergo early impairment related to AD

[71, 72]. Cortical nAChRs, as assessed in vivo using ^{11}C -nicotine binding in patients with mild AD, are robustly associated with attention cognitive function [73]. Moreover, the olfactory identification score was negatively correlated with regional cerebral blood flow in several brain areas including the bilateral frontal pole, in patients with mild cognitive impairment (MCI) and AD [74].

Since the $\alpha 4\beta 2$ -like nAChRs in the brain decline with age as well as in AD, activation of the nAChRs involved in cortical vasodilatation or potentiation of olfactory bulb vasodilation could be beneficial for older people and AD patients. To this end, administering nicotinic receptor agonists or physical therapies such as somatosensory stimulation and walking, known to activate basal forebrain cholinergic system in both adult and old rats [75–80], may have therapeutic values. Furthermore, the increased cerebral blood flow in the neocortex and olfactory bulb induced by $\alpha 4\beta 2$ -like nAChR activation could improve oxygen and glucose delivering to those brain areas, and those sufficient nourishments appear to be beneficial for neuronal protection and maintaining cognitive function and olfaction.

Abbreviations

AD	Alzheimer's disease
HDB	Horizontal limb of the diagonal band of Broca
MCI	Mild cognitive impairment
MS	Medial septal nucleus
NBM	Nucleus basalis of Meynert
SI	Substantia innominate
VDB	Ventral limb of the diagonal band of Broca
nAChR	Nicotinic acetylcholine receptor

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References

- Doty RL, Shaman P, Applebaum SL, Giberson R, Siksorski L, Rosenberg L (1984) Smell identification ability: changes with age. *Science* 226:1441–1443
- Devanand DP, Liu X, Tabert MH, Pradhaban G, Cuasay K, Bell K, de Leon MJ, Doty RL, Stern Y, Pelton GH (2008) Combining early markers strongly predicts conversion from mild cognitive impairment to Alzheimer's disease. *Biol Psychiatry* 64:871–879
- Murphy C (2019) Olfactory and other sensory impairments in Alzheimer disease. *Nat Rev Neurol* 15:11–24
- Mesulam MM, Mufson EJ, Wainer BH, Levey AI (1983) Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1–Ch6). *Neuroscience* 10:1185–1201
- Rye DB, Wainer BH, Mesulam MM, Mufson EJ, Saper CB (1984) Cortical projections arising from the basal forebrain: a study of cholinergic and noncholinergic components employing combined retrograde tracing and immunohistochemical localization of choline acetyltransferase. *Neuroscience* 13:627–643
- Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, DeLong MR (1982) Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science* 215:1237–1239
- Lehéricy S, Hirsch EC, Cervera-Piéro P, Hersh LB, Bakchine S, Piette F, Duyckaerts C, Hauw JJ, Javoy-Agid F, Agid Y (1993) Heterogeneity and selectivity of the degeneration of cholinergic neurons in the basal forebrain of patients with Alzheimer's disease. *J Comp Neurol* 330:15–31
- Grothe M, Heinsen H, Teipel SJ (2012) Atrophy of the cholinergic basal forebrain over the adult age range and in early stages of Alzheimer's disease. *Biol Psychiatry* 71:805–813
- Grothe M, Zaborszky L, Atienza M, Gil-Neciga E, Rodriguez-Romero R, Teipel SJ, Amunts K, Suarez-Gonzalez A, Cantero JL (2010) Reduction of basal forebrain cholinergic system parallels cognitive impairment in patients at high risk of developing Alzheimer's disease. *Cereb Cortex* 20:1685–1695
- Luiten PGM, Gaykema RPA, Traber J, Spencer DG Jr (1987) Cortical projection patterns of magnocellular basal nucleus subdivisions as revealed by anterogradely transported *Phaseolus vulgaris* leucoagglutinin. *Brain Res* 413:229–250
- Biesold D, Inanami O, Sato A, Sato Y (1989) Stimulation of the nucleus basalis of Meynert increases cerebral cortical blood flow in rats. *Neurosci Lett* 98:39–44
- Hotta H (2016) Neurogenic control of parenchymal arterioles in the cerebral cortex. *Prog Brain Res* 225:3–39
- Sato A, Sato Y (1992) Regulation of regional cerebral blood flow by cholinergic fibers originating in the basal forebrain. *Neurosci Res* 14:242–274
- Nordberg A, Alafuzoff I, Winblad B (1992) Nicotinic and muscarinic subtypes in the human brain: changes with aging and dementia. *J Neurosci Res* 31:103–111
- Geula C, Dunlop SR, Ayala I, Kawles AS, Flanagan ME, Gefen T, Mesulam MM (2021) Basal forebrain cholinergic system in the dementias: vulnerability, resilience, and resistance. *J Neurochem* 158:1394–1411
- Crystal GJ, Downey HF, Adkins TP, Bashour FA (1983) Regional blood flow in canine brain during nicotine infusion: effect of autonomic blocking drugs. *Stroke* 14:941–947
- Linville DG, Williams S, Raszkievicz JL, Arneric SP (1993) Nicotinic agonists modulate basal forebrain control of cortical cerebral blood flow in anesthetized rats. *J Pharmacol Exp Ther* 267:440–448
- Uchida S, Kagitani F, Nakayama H, Sato A (1997) Effect of stimulation of nicotinic cholinergic receptors on cortical cerebral blood flow and changes in the effect during aging in anesthetized rats. *Neurosci Lett* 228:203–206
- Hotta H, Uchida S, Kagitani F, Maruyama N (2011) Control of cerebral cortical blood flow by stimulation of basal forebrain cholinergic areas in mice. *J Physiol Sci* 61:201–209
- Uchida S, Kawashima K, Lee TJ (2002) Nicotine-induced NO-mediated increase in cortical cerebral blood flow is blocked by beta2-adrenoceptor antagonists in the anesthetized rats. *Auton Neurosci* 96:126–130
- Azam L, Winzer-Serhan U, Leslie FM (2003) Co-expression of alpha7 and beta2 nicotinic acetylcholine receptor subunit mRNAs within rat brain cholinergic neurons. *Neuroscience* 119:965–977
- Gaimarri A, Moretti M, Riganti L, Zanardi A, Clementi F, Gotti C (2007) Regulation of neuronal nicotinic receptor traffic and expression. *Brain Res Rev* 55:134–143
- Piccio MR, Caldarone BJ, Brunzell DH, Zachariou V, Stevens TR, King SL (2001) Neuronal nicotinic acetylcholine receptor subunit knockout mice: physiological and behavioral phenotypes and possible clinical implications. *Pharmacol Ther* 92:89–108
- Uchida S, Hotta H, Kawashima K (2009) Long-term nicotine treatment reduces cerebral cortical vasodilation mediated by alpha4beta2-like nicotinic acetylcholine receptors in rats. *Eur J Pharmacol* 609:100–104
- Nott A, Levin ED (2006) Dorsal hippocampal alpha7 and alpha4beta2 nicotinic receptors and memory. *Brain Res* 1081:72–78
- Wang Y, Sherwood JL, Miles CP, Whiffin G, Lodge D (2006) TC-2559 excites dopaminergic neurones in the ventral tegmental area by stimulating alpha4beta2-like nicotinic acetylcholine receptors in anaesthetised rats. *Br J Pharmacol* 147:379–390
- Harvey SC, Maddox FN, Luetje CW (1996) Multiple determinants of dihydro-beta-erythroidine sensitivity on rat neuronal nicotinic receptor alpha subunits. *J Neurochem* 67:1953–1959
- Dwoskin LP, Crooks PA (2001) Competitive neuronal nicotinic receptor antagonists: a new direction for drug discovery. *J Pharmacol Exp Ther* 298:395–402
- Xiao Y, Kellar KJ (2004) The comparative pharmacology and up-regulation of rat neuronal nicotinic receptor subtype binding sites stably expressed in transfected mammalian cells. *J Pharmacol Exp Ther* 310:98–107
- Sato A, Sato Y, Uchida S (2002) Regulation of cerebral cortical blood flow by the basal forebrain cholinergic fibers and aging. *Auton Neurosci* 96:13–19
- Uchida S, Hotta H (2009) Cerebral cortical vasodilatation mediated by nicotinic cholinergic receptors: effects of old age and of chronic nicotine exposure. *Biol Pharm Bull* 32:341–344
- Araujo DM, Lapchak PA, Meaney MJ, Collier B, Quirion R (1990) Effects of aging on nicotinic and muscarinic autoreceptor function in the rat brain: relationship to presynaptic cholinergic markers and binding sites. *J Neurosci* 10:3069–3078
- Shiba K, Machida T, Uchida S, Hotta H (2006) Effects of nicotine on regional blood flow in the olfactory bulb in rats. *Eur J Pharmacol* 546:148–151
- Hamamoto M, Kiyokage E, Sohn J, Hioki H, Harada T, Toida K (2017) Structural basis for cholinergic regulation of neural circuits in the mouse olfactory bulb. *J Comp Neurol* 525:574–591
- Zaborszky L, Carlsen J, Brashear HR, Heimer L (1986) Cholinergic and GABAergic afferents to the olfactory bulb in the rat with special emphasis on the projection neurons in the nucleus of the horizontal limb of the diagonal band. *J Comp Neurol* 243:488–509
- Uchida S, Kagitani F (2018) Effect of basal forebrain stimulation on extracellular acetylcholine release and blood flow in the olfactory bulb. *J Physiol Sci* 68:415–423
- Boido D, Rungta RL, Osmanski BF, Roche M, Tsurugizawa T, Le Bihan D, Ciobanu L, Charpak S (2019) Mesoscopic and microscopic imaging of sensory responses in the same animal. *Nat Commun* 10:1110. <https://doi.org/10.1038/s41467-019-09082-4>
- Chaigneau E, Tiret P, Lecoq J, Ducros M, Knöpfel T, Charpak S (2007) The relationship between blood flow and neuronal activity in the rodent olfactory bulb. *J Neurosci* 27:6452–6460
- Iadecola C (2017) The neurovascular unit coming of age: a journey through neurovascular coupling in health and disease. *Neuron* 96:17–42
- Poplawsky AJ, Fukuda M, Murphy M, Kim SG (2015) Layer-specific fMRI responses to excitatory and inhibitory neuronal activities in the olfactory bulb. *J Neurosci* 35:15263–15275
- Hosford PS, Gourine AV (2019) What is the key mediator of the neurovascular coupling response? *Neurosci Biobehav Rev* 96:174–181
- Petzold GC, Albeanu DF, Sato TF, Murthy VN (2008) Coupling of neural activity to blood flow in olfactory glomeruli is mediated by astrocytic pathways. *Neuron* 58:897–910

43. Uchida S, Ito Y, Kagitani F (2019) Effects of nicotine on odor-induced increases in regional blood flow in the olfactory bulb in rats. *J Physiol Sci* 69:425–431
44. Connelly T, Savigner A, Ma M (2013) Spontaneous and sensory-evoked activity in mouse olfactory sensory neurons with defined odorant receptors. *J Neurophysiol* 110:55–62
45. Duchamp-Viret P, Chaput MA, Duchamp A (1999) Odor response properties of rat olfactory receptor neurons. *Science* 284(5423):2171–2174
46. Reisert J, Matthews HR (2001) Response properties of isolated mouse olfactory receptor cells. *J Physiol* 530(Pt 1):113–122
47. Uchida S, Kagitani F (2020) Effects of nicotine on regional blood flow in the olfactory bulb in response to olfactory nerve stimulation. *J Physiol Sci* 70:30. <https://doi.org/10.1186/s12576-020-00758-x>
48. Bendahmane M, Ogg MC, Ennis M, Fletcher ML (2016) Increased olfactory bulb acetylcholine bi-directionally modulates glomerular odor sensitivity. *Sci Rep* 6:25808. <https://doi.org/10.1038/srep25808>
49. D'Souza RD, Vijayaraghavan S (2014) Paying attention to smell: cholinergic signaling in the olfactory bulb. *Front Synaptic Neurosci* 6:21. <https://doi.org/10.3389/fnsyn.2014.00021>
50. Burton SD, LaRocca G, Liu A, Cheetham CE, Urban NN (2017) Olfactory bulb deep short-axon cells mediate widespread inhibition of tufted cell apical dendrites. *J Neurosci* 37:1117–1138
51. Spindle MS, Parsa PV, Bowles SG, D'Souza RD, Vijayaraghavan S (2018) A dominant role for the beta 4 nicotinic receptor subunit in nicotinic modulation of glomerular microcircuits in the mouse olfactory bulb. *J Neurophysiol* 120:2036–2048
52. Keiger CJ, Walker JC (2000) Individual variation in the expression profiles of nicotinic receptors in the olfactory bulb and trigeminal ganglion and identification of alpha2, alpha6, alpha9, and beta3 transcripts. *Biochem Pharmacol* 59:233–240
53. Wada E, Wada K, Boulter J, Deneris E, Heinemann S, Patrick J, Swanson LW (1989) Distribution of alpha 2, alpha 3, alpha 4, and beta 2 neuronal nicotinic receptor subunit mRNAs in the central nervous system: a hybridization histochemical study in the rat. *J Comp Neurol* 284:314–335
54. Uchida S, Moriya J, Morihara D, Kagitani F (2023) Nicotinic cholinergic regulation of olfactory bulb blood flow response in aged rats. *J Physiol Sci* 73:1. <https://doi.org/10.1186/s12576-022-00859-9>
55. Kass MD, Czarniecki LA, McGann JP (2018) Stable olfactory sensory neuron in vivo physiology during normal aging. *Neurobiol Aging* 69:33–37
56. Ferrari R, Pedrazzi P, Algeri S, Agnati LF, Zoli M (1999) Subunit and region-specific decreases in nicotinic acetylcholine receptor mRNA in the aged rat brain. *Neurobiol Aging* 20:37–46
57. Mitsis EM, Cosgrove KP, Staley JK, Bois F, Frohlich EB, Tamagnan GD, Estok KM, Seibyl JP, van Dyck CH (2009) Age-related decline in nicotinic receptor availability with [¹²³I]5-IA-85380 SPECT. *Neurobiol Aging* 30:1490–1497
58. Tohgi H, Utsugisawa K, Yoshimura M, Nagane Y, Mihara M (1998) Age-related changes in nicotinic acetylcholine receptor subunits alpha4 and beta2 messenger RNA expression in postmortem human frontal cortex and hippocampus. *Neurosci Lett* 245:139–142
59. Sabri O, Meyer PM, Gräf S, Hesse S, Wilke S, Becker GA, Rullmann M, Patt M, Luthardt J, Wagenknecht G, Hoepping A, Smits R, Franke A, Sattler B, Tjepolt S, Fischer S, Deuther-Conrad W, Hegerl U, Barthel H, Schönknecht P, Brust P (2018) Cognitive correlates of alpha2B nicotinic acetylcholine receptors in mild Alzheimer's dementia. *Brain* 141:1840–1854
60. Ohata M, Sundaram U, Fredericks WR, London ED, Rapoport SI (1981) Regional cerebral blood flow during development and ageing of the rat brain. *Brain* 104:319–332
61. Salter JM, Cassone VM, Wilkerson MK, Delp MD (1998) Ocular and regional cerebral blood flow in aging Fischer-344 rats. *J Appl Physiol* 85:1024–1029
62. Shaw TG, Mortel KF, Meyer JS, Rogers RL, Hardenberg J, Cutaja MM (1984) Cerebral blood flow changes in benign aging and cerebrovascular disease. *Neurology* 34:855–862
63. Pantano P, Baron JC, Lebrun-Grandié P, Duquesnoy N, Bousser MG, Comar D (1984) Regional cerebral blood flow and oxygen consumption in human aging. *Stroke* 15:635–641
64. Leidhin CN, McMorrow J, Carey D, Newman L, Williamson W, Fagan AJ, Chappell MA, Kenny RA, Meaney JF, Knight SP (2021) Age-related normative changes in cerebral perfusion: data from the Irish longitudinal study on ageing (TILDA). *Neuroimage* 229:117741
65. Attems J, Walker L, Jellinger KA (2015) Olfaction and aging: a mini-review. *Gerontology* 61:485–490
66. Schubert CR, Fischer ME, Pinto AA, Klein BEK, Klein R, Cruickshanks KJ (2017) Odor detection thresholds in a population of older adults. *Laryngoscope* 127:1257–1262
67. Uchida S, Shimada C, Sakuma N, Kagitani F, Kan A, Awata S (2020) The relationship between olfaction and cognitive function in the elderly. *J Physiol Sci* 70:48. <https://doi.org/10.1186/s12576-020-00777-8>
68. Uchida S, Shimada C, Sakuma N, Kagitani F, Kan A, Awata S (2022) Olfactory function and discrimination ability in the elderly: a pilot study. *J Physiol Sci* 72:8. <https://doi.org/10.1186/s12576-022-00832-6>
69. Baxter MG, Chiba AA (1999) Cognitive functions of the basal forebrain. *Curr Opin Neurobiol* 9:178–183
70. Schilit Nitenson A, Manzano Nieves G, Poeta DL, Bahar R, Rachofsky C, Mandairon N, Bath KG (2019) Acetylcholine regulates olfactory perceptual learning through effects on adult neurogenesis. *iScience* 22:544–556
71. Perry RJ, Hodges JR (1999) Attention and executive deficits in Alzheimer's disease. A critical review. *Brain* 122:383–404
72. Yang J, Ogasa T, Ohta Y, Abe K, Wu J (2010) Decline of human tactile angle discrimination in patients with mild cognitive impairment and Alzheimer's disease. *J Alzheimers Dis* 22:225–234
73. Kadir A, Almkvist O, Wall A, Långström B, Nordberg A (2006) PET imaging of cortical ¹¹C-nicotine binding correlates with the cognitive function of attention in Alzheimer's disease. *Psychopharmacology* 188:509–520
74. Kashibayashi T, Takahashi R, Fujita J, Fujito R, Kamimura N, Okutani F, Kazui H (2021) Correlation between cerebral blood flow and olfactory function in mild cognitive impairment and Alzheimer's disease. *Int J Geriatr Psychiatry* 36:1103–1109
75. Akaishi T, Kimura A, Sato A, Suzuki A (1990) Responses of neurons in the nucleus basalis of Meynert to various afferent stimuli in rats. *NeuroReport* 1:37–39
76. Kimura A, Okada K, Sato A, Suzuki H (1994) Regional cerebral blood flow in the frontal, parietal and occipital cortices increases independently of systemic arterial pressure during slow walking in conscious rats. *Neurosci Res* 20:309–315
77. Kurosawa M, Sato A, Sato Y (1992) Cutaneous mechanical sensory stimulation increases extracellular acetylcholine release in cerebral cortex in anesthetized rats. *Neurochem Int* 21:423–427
78. Kurosawa M, Okada K, Sato A, Uchida S (1993) Extracellular release of acetylcholine, noradrenaline and serotonin increases in the cerebral cortex during walking in conscious rats. *Neurosci Lett* 161:73–76
79. Uchida S, Kagitani F, Suzuki A, Aikawa Y (2000) Effect of acupuncture-like stimulation on cortical cerebral blood flow in anesthetized rats. *Jpn J Physiol* 50:495–507
80. Uchida S, Kagitani F (2015) Effect of acupuncture-like stimulation on cortical cerebral blood flow in aged rats. *J Physiol Sci* 65:67–75

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