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EFFECTS OF NORMAL AGING ON PREFRONTAL AREA 46 IN THE RHESUS MONKEY

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Abstract

This review is concerned with the effects of normal aging on the structure and function of prefrontal area 46 in the rhesus monkey (*Macaca mulatta*). Area 46 has complex connections with somatosensory, visual, visuomotor, motor and limbic systems and a key role in cognition, which frequently declines with age. An important question is what alterations might account for this decline. We are nowhere near having a complete answer, but as will be shown in this review, it is now evident that there is no single underlying cause. There is no significant loss of cortical neurons and although there are a few senile plaques in rhesus monkey cortex, their frequency does not correlate with cognitive decline. However, as discussed in this review, the following do correlate with cognitive decline. Loss of white matter, that has been proposed to result in some disconnections between parts of the central nervous system, and changes in the structure of myelin sheaths, that reduce conduction velocity and the timing in neuronal circuits. In addition, there are reductions in the inputs to cortical neurons, as shown by regression of dendritic trees, loss of dendritic spines and synapses, and alterations in transmitters and receptors. These factors contribute to alterations in the intrinsic and network physiological properties of cortical neurons. As more details emerge it is to be hoped that effective interventions to retard cognitive decline can be proposed.

Keywords

Frontal cortex; primate; cognition; working memory; Macaque

1. Overview

This review is concerned with the effects of normal aging on the structure and function of prefrontal area 46 in the rhesus monkey. Even though more work needs to be done, in terms of normal aging area 46 is probably the most completely studied of the neocortical areas involved in the mediation of cognitive abilities such as working memory. Rhesus monkeys are an ideal primate model for studying normal aging for several reasons: they have a maximum lifespan of 35 years (Tigges et al., 1988) but are not subject to Alzheimer's disease, their brains

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do not acquire neurofibrillary tangles and they have few senile plaques in the cortex. Since humans can live for about one hundred years, and the life-span of monkeys is about 35 years, as a rule of thumb it can be assumed that one monkey year is approximately equal to three human years. This also fits with the fact that monkeys are sexually mature at 5 years of age, and humans at about 13–15 years of age. Another advantage of studying monkey is that over their entire life-span monkeys can be behaviorally tested to determine their cognitive status, and then their brains prepared for anatomical and other assessments. Consequently it can be determined if age-related decline in cognitive status can be correlated with any of the morphological, physiological, or biochemical alterations that occur with age. For example, the few senile plaques present have a predilection for the frontal and primary somatosensory cortices in monkeys (Struble et al., 1985; Heilbroner and Kemper, 1990; Sloane et al., 1997), and their numbers increase with age, as they do in humans. However, although both plaque burden and cognitive decline tend to increase with age, there is no correlation between plaque density and the extent of age-related behavioral dysfunction (Sloane et al., 1997). Incidentally, in healthy humans as in monkeys, the predominant form of amyloid is A β ₄₀, but in Alzheimer's disease, which monkeys do not get, the prevalence of A β ₄₂ is increased (Gearing et al., 1996; Kanemaru et al., 1996). This likely means that the processing of amyloid is different in humans and monkeys, but at present the significance of this fact is not evident, although it may relate to the low plaque frequency in monkeys.

Prefrontal area 46, specifically its central and caudal parts, has been associated with working memory since the classic work of Jacobsen (Jacobsen, 1936). This cortical area has a critical role in a range of cognitive tasks that require keeping in mind information on a temporary basis to accomplish a task at hand, or to keep track of self-generated responses to complete tasks with several components, such as following road directions, or a recipe [for reviews see (Goldman-Rakic, 1988; Fuster, 1989; Fuster, 1993; Funahashi and Takeda, 2002)]. In non-human primates the role of area 46 in working memory has been shown through delayed response tasks using a variety of sensory stimuli and requiring a variety of responses involving hand movement to retrieve a reward, oculomotor responses, or delayed alternation responses [e.g., (Carlson et al., 1997)]. Damage to area 46 in non-human primates creates a blind spot specific for temporary memory, and impairs the ability to accomplish the task at hand (Jacobsen, 1936; Funahashi et al., 1993), leaving other types of memory intact. The simplicity of the tasks that are disrupted belies the complexity of information necessary to accomplish them. As will be shown in a later section, area 46 communicates with a host of cortical and subcortical structures that makes it possible to use information flexibly for cognitive operations.

2. Age-related changes in area 46-mediated cognitive abilities in the rhesus monkey

As described above, area 46 plays a critical role in the mediation of executive functions, including working memory. These executive functions, essential for accomplishing tasks of daily living, are among the earliest cognitive abilities to decline during normal aging in both humans (Albert, 1993; Salthouse et al., 2003; Fisk and Sharp, 2004; Rhodes, 2004; Rodriguez-Aranda and Sundet, 2006; Sorel and Pennequin, 2008) and non-human primates (Bartus et al., 1979; Rapp, 1990; Lai et al., 1995a; Steere and Arnsten, 1997; Herndon et al., 1997; Voytko, 1999; Moore et al., 2003; Moore et al., 2005; Moore et al., 2006).

In the rhesus monkey, executive function has been assessed primarily with visual reversal learning tasks (both spatial and object modalities). In order to succeed at these tasks, monkeys must be able to shift from an initial learned stimulus-reinforcement pair to a different stimulus-reinforcement pair. Performance on reversal learning tasks provides a measure of set-shifting ability, which is an indication of cognitive flexibility. Impairment on reversal learning tasks is

manifested both as an inability to shift set and as an increase in perseveration. Bartus and colleagues were the first to demonstrate significant impairment of reversal learning in aged rhesus monkeys (Bartus et al., 1979). Several years later there was a report that initial learning of the reversal learning task required more training in aged compared to young monkeys, while overall reversal learning was largely intact, leading to the idea that attentional deficits during task acquisition contribute significantly to cognitive deficits in aged monkeys (Rapp, 1990). By contrast, another study reported that there is no difference between aged and young subjects in initial learning of spatial and object reversal tasks, but found that aged monkeys as a group are significantly impaired on spatial but not object reversals (Lai et al., 1995a). Importantly, these investigators also noted a distinct perseverative tendency of aged monkeys on both spatial and object reversals (Lai et al., 1995a).

In one comprehensive study of cognitive decline in aging rhesus monkeys, the performance of early (19–23 years old), advanced (24–28 years old), and oldest (≥ 29 years old) aged monkeys were compared to that of young adults (< 15 years old) on a variety of tasks that test cognitive and mnemonic function, including: (1) acquisition of the delayed non-matching-to-sample task (DNMS); (2) performance of the DNMS with a delay of 120 sec; (3) the spatial condition of the delayed recognition span test (DRST); (4) the color condition of the DRST; (5) spatial reversal learning; (6) object reversal learning; and (7) a composite Cognitive Impairment Index (CII) score which was derived from the average of the three standardized scores DNMS basic task, the DNMS 2-minute delay and DRST spatial condition using the guidance of a principal components analysis (Herndon et al., 1997). Both early-aged and oldest-aged monkeys were significantly impaired on all tasks except on the DRST-color, and the highest degree of impairment was seen in spatial memory (DRST). Progressively higher impairment rates were seen with increasing age for DNMS-acquisition, DRST-color and spatial reversal learning tasks and the CII. Recently, a new task termed the Conceptual Set Shifting Task, has been developed and employed to assess abstraction, concept formation and set shifting in monkeys in a similar way to the Wisconsin Card Sorting Test used in human studies (Moore et al., 2005). Aged monkeys (> 20 years old) are significantly impaired in the acquisition and performance on the Conceptual Set Shifting Task and also exhibit a high degree of perseverative responding relative to young adult monkeys (5–10 years of age; (Moore et al., 2005).

There is thus now a relatively large body of literature demonstrating that aged monkeys are impaired in executive function relative to young monkeys. With this established, it is important to determine the age at which this dysfunction begins. A recent study determined that middle-aged monkeys (10 to 20 years old) are significantly impaired on the Conceptual Set Shifting Task as are the aged subjects (Moore et al., 2006). These findings are consistent with those of human studies which have shown a significant increase in perseverative errors on the Wisconsin Card Sorting Test by middle-age (Rhodes, 2004).

Taken together, these behavioral studies support the idea that in the rhesus monkey executive function (mediated in part by area 46) undergoes significant decline fairly early during the aging process. However, in terms of cognitive impairment within any aged population of monkeys, there is a clear gradient of cognitive performance, with many aged monkeys being impaired while others are unimpaired and indistinguishable from young monkeys. Hence, as with the human population, some aged monkeys are “unsuccessful agers” and some are cognitively spared and are “successful agers”.

3. Architecture of area 46

Area 46 is situated in the center of the lateral surface of the forebrain in macaque monkeys and humans. In macaque monkeys it occupies the banks of the principal sulcus nearly throughout the entire extent of the sulcus, except at its rostral and caudal tips, which are occupied by area

10 in the frontal pole, and area 8 in front of the concavity of the arcuate sulcus (Fig. 1). Beyond the principal sulcus, area 46 extends to the gyral surface above, where it shares a border with area 9, and below the sulcus it abuts area 12.

In Nissl stained sections, area 46 has the appearance of a typical granular cortex, since layer 4 contains small and closely packed neurons (Fig. 1 B, C). However, layer 4 is of variable thickness, so that its upper border with layer 3 is not always well defined. The neurons in layer 3 are larger than those in layer 4, and there is a scattering of quite large, darkly staining pyramidal neurons in the lower portion of layer 3. Towards the upper part of layer 3 the neurons gradually become smaller. Layer 2 is composed of small neurons and has a well-delineated border with layer 1, which contains few neurons. The border between layer 4 and layer 5 is also irregular, but it is conspicuous because layer 5 contains large and medium sized pyramidal neurons that are more loosely packed than the small neurons of layer 4. Defining the exact border between layer 5 and layer 6A can be difficult, although the neurons of layer 6A tend to be more rounded than the pyramidal shaped neurons of layer 5. In contrast, the border between layer 6A and 6B is easily discerned because the neurons in layer 6B are mainly horizontal neurons, and they intermix with the myelinated fibers of the upper strata of the white matter (O'Donnell et al., 1999).

Area 46 can be subdivided into anterior and posterior sectors, and each sector is further subdivided into a dorsal and a ventral part (Fig. 1A). There is general agreement on the overall extent of area 46 in rhesus monkeys, though not for the number or borders of its more subtle subdivisions. On the basis of the neuronal architecture, the various maps generally agree that the cortex buried in the principal sulcus is part of area 46 (Walker, 1940; Barbas and Pandya, 1989; Preuss and Goldman-Rakic, 1991; Petrides and Pandya, 1999), as is its small extension above and below the principal sulcus. In a recent study the caudal extension of area 46 into the adjacent gyri has been renamed 9/46d (above the principal sulcus) and 9/46v (below the principal sulcus) in an attempt to make it consistent with terminology used for the human (Petrides and Pandya, 1999). The architectonic boundaries of the various subdivisions are subtle, and the most prominent architectonic difference, detected using quantitative architectonic methods, is a higher density of the calcium binding protein calbindin in rostral compared to caudal area 46 (Dombrowski et al., 2001).

According to the analysis carried out by Petrides and Pandya (1999), by comparison to the monkey, in the human brain area 46 occupies the middle portion of the middle frontal gyrus, with a considerable portion of the area buried in the depths of the middle frontal sulcus. Petrides and Pandya (1999) state that only a constricted portion of what had been previously labeled as area 46 in the monkey brain has the same cytological characteristics as area 46 in the human brain, and for a full discussion of the complexities involved in considering the homologies between the human and monkey prefrontal cortices it is recommended that the article by Petrides and Pandya (1999) be consulted.

4. Effects of age on volume, neuronal numbers, and minicolumns

On the basis of early studies of the effects of age on cerebral cortex it was assumed that any loss of cognition with age is due to a substantial loss of neurons from the cerebral cortex. However, as more rigorous studies have been carried out, it has become evident there is no significant age-related loss of cortical neurons during normal aging and that the erroneous conclusions of earlier investigators were due to problems in counting techniques (Peters et al., 1998a). The question of whether there is loss of neurons from area 46 of monkey prefrontal cortex has been thoroughly examined in the past few years. The issue was first addressed by Peters et al. (Peters et al., 1994), who found no change in the numbers of neuronal profiles in strips of sections extending through the depth of area 46, and no indication that neurons are

dying. More recently, Smith et al. (Smith et al., 2004) have also examined the effects of age on neuronal numbers in both areas 46 and 8A of behaviorally tested monkeys and on the basis of a stereological analysis they agree that there is no decline in the total numbers of neurons in area 46 with age. Surprisingly, however, they did find a 32% loss of neurons from adjacent area 8A with age, leading them to conclude that any age-related loss of neurons from the cortex is focal.

Other studies have applied stereological methods to estimate the volume of area 46 in Nissl stained sections, and found no change with age, indicating that a loss of grey matter from this cortical area is not the basis of age-related behavioral deficits (O'Donnell et al., 1999). A recent magnetic resonance imaging (MRI) study on the effects of age on gray matter of rhesus monkey cortex however reports a small loss of gray matter from several cortical areas, including the dorsolateral prefrontal cortex, where area 46 is located, and concludes that the loss of gray matter is associated with the decline in working memory exhibited by the monkeys (Alexander et al., 2008). This loss of gray matter amounts to about a 13% thinning of the cortex (Alexander et al., 2008), but in earlier studies in which the thickness of the cortex of area 46 was measured in Nissl stained sections, no change in thickness was found with age, and so the true state of affairs is unclear (Peters et al., 1994; (O'Donnell et al., 1999). However, it can probably be concluded that any decrease in the thickness of the cortex with age is minimal, except perhaps for layer 1. In area 46 the width of layer 1 decreases by about 25% (as assessed using 1 μ m sections), although there is no loss of neurons from layer 1 with age (Peters and Sethares, 2002b). The thinning is accompanied by a loss of dendrites and dendritic spines from the apical tufts of pyramidal neurons (Peters et al., 1998b), and interestingly, the thinning correlates with a deficit in the performance of monkeys in a delayed non-matching to sample task. However, contrary to these observations, O'Donnell et al. (O'Donnell et al., 1999) subsequently measured the thickness of layer 1 as part of their study of the volume of area 46, and found no decrease in the thickness of layer 1 in Nissl stained sections with age, perhaps because of the lack of sharpness in laminar boundaries in the Nissl preparation.

According to a recent digitized neuronal density map analysis (Cruz et al., 2004), the neurons in area 46 are arranged in vertically oriented minicolumns that are each about 22 microns wide. A correlative in-depth morphological analysis has not been carried out, but it is likely that, as in other cortices, the apical dendrites of the pyramidal neurons in layers 5 through 2 are clustered together as they ascend through the cortex, and the myelinated efferent nerve fibers of the pyramidal neurons are aggregated into bundles as they descend towards the white matter [e.g., see (Peters, 1996)]. The digitized density map probably reflects the dimensions of the center-to-center spacing between the apical dendritic clusters. Cruz et al. (Cruz et al., 2004) also report that with increasing age the integrity of the minicolumns is compromised, because statistically there is significant decrease in the "strength" of minicolumns, indicating that neurons are being randomly displaced by as much as 3 μ m. Interestingly this change significantly correlates with age-related cognitive decline.

5. Unique connections of the subsectors of area 46

The cognitive functions of area 46 are likely supported by the rich projections it receives from a host of cortical and subcortical structures, as highlighted here. The major sources of monosynaptic connections of area 46 are from other cortical areas. Area 46 is connected with unimodal visual, auditory and somatosensory association cortices but not directly with olfactory cortices. The different sectors of area 46 are differentially connected with each of the above association cortices, as highlighted below and in Figure 2.

Connections with visual and visuomotor cortices

One of the major sources of connections of area 46 is the visual cortical association system, which involves specifically the caudal and ventral part of area 46 (Jones and Powell, 1970; Chavis and Pandya, 1976; Jacobson and Trojanowski, 1977; Barbas and Mesulam, 1985; Barbas, 1988; Webster et al., 1994), originate mostly from the inferior temporal cortex (areas TE2–3, TEa and TEm), and to a lesser extent from areas V4, V3, TEO and MT (Barbas and Mesulam, 1985; Barbas, 1988; Webster et al., 1994), in connections that are reciprocal (Rempel-Clower and Barbas, 2000). Caudal area 46 is also strongly connected with the lateral (ventral) bank of the intraparietal sulcus, which has visual and visuomotor properties (Barbas, 1988; Medalla and Barbas, 2006). These rich connections appear to be critical in cognitive operations and in orienting to visual stimuli that are relevant to the task at hand (Savaki and Dalezios, 1999; Moschovakis et al., 2004). Moreover, area 46 and the ventral intraparietal cortex are co-activated in visuomotor functions (Friedman and Goldman-Rakic, 1994).

Connections with auditory association cortices

Area 46, involving mostly its caudal half, is bidirectionally connected with superior temporal auditory association areas Ts2–3 and TAa, which overlap with areas known as the parabelt (Barbas and Mesulam, 1985; Petrides and Pandya, 1988; Hackett et al., 1999; Romanski et al., 1999a). A mid-dorsal part of area 46 is a major focus of connections with auditory association areas (Barbas and Mesulam, 1985; Hackett et al., 1999; Romanski et al., 1999b), as well as the dorsal part of rostral area 46, which receives projections from auditory association areas Ts1–Ts3 and Tpt (Petrides and Pandya, 1999), in pathways that are reciprocal (Medalla et al., 2007). The projections from prefrontal areas to auditory association areas may provide the circuit for selecting relevant auditory information and suppressing distracters, through association with laminar-specific neurochemical and functional classes of inhibitory neurons in auditory association cortex (Barbas et al., 2005; Medalla et al., 2007). Behavioral findings indicate that humans with dorsolateral prefrontal lesions make more errors in auditory discrimination tasks, specifically in the presence of distracting stimuli (Chao and Knight, 1998). Similar findings are reported for aged humans, suggesting that changes in the pathway from dorsolateral prefrontal cortex to auditory association cortex compromise the ability to ignore irrelevant signals (Chao and Knight, 1997).

Connections with somatosensory cortices

Another major source of cortical connections is from somatosensory association cortices, directed to the central part of ventral area 46 (Jones and Powell, 1970; Chavis and Pandya, 1976; Barbas and Mesulam, 1985; Barbas, 1988; Preuss and Goldman-Rakic, 1989; Cavada and Goldman-Rakic, 1989). The principal sources within somatosensory cortex include rostral intraparietal area PF (or area 7b), area SII, and areas 1 and 2 (Petrides and Pandya, 1984; Barbas and Mesulam, 1985; Cavada and Goldman-Rakic, 1989; Cipolloni and Pandya, 1999; Lewis and Van Essen, 2000). Sparse projections from the superior parietal lobule (area PE) terminate in the posterior half of dorsal area 46 (Petrides and Pandya, 1984; Morecraft et al., 2004), as do projections from the medial part of area 7 (Cavada and Goldman-Rakic, 1989). The rostro-ventral part of area 46 receives projections from the gustatory region, as well as from somatosensory areas 1, 2 and SII in the pericentral operculum (Cipolloni and Pandya, 1999).

Connections with polymodal areas

Area 46 is also connected with polymodal areas, including the upper (medial) bank of the superior temporal sulcus, where neurons respond to auditory, visual and/or somatosensory stimuli (Seltzer and Pandya, 1978). Throughout its extent, area 46 has bidirectional connections

with these polymodal temporal cortices (Barbas and Mesulam, 1985; Barbas, 1988; Seltzer and Pandya, 1989; Petrides and Pandya, 1999).

Connections of area 46 within the prefrontal cortices

Area 46 is also connected monosynaptically with neighboring prefrontal cortices, possibly gaining access to signals that do not reach its individual sectors directly. Rostral area 46 is connected with frontal polar area 10 (Carmichael and Price, 1996), particularly its dorsal sector (Barbas and Mesulam, 1985; Barbas and Pandya, 1989), and with area 9 (Barbas and Mesulam, 1985; Barbas and Pandya, 1989), where it targets both excitatory and inhibitory neurons (Medalla and Barbas, 2009). Ventral area 46 is also innervated by area 9 (Pucak et al., 1996; Melchitzky et al., 1998). The caudal sector of area 46 has extensive bidirectional connections with adjacent area 8 (Barbas and Mesulam, 1981; Preuss and Goldman-Rakic, 1985; Barbas, 1988; Barbas and Pandya, 1989). Ventral area 46 has bidirectional connections with area 12 and orbitofrontal area 11 (Barbas and Mesulam, 1985; Barbas, 1988; Barbas and Pandya, 1989).

Connections with limbic cortices

Area 46 has significant connections with cortical limbic structures, broadly associated with memory processes, emotions, and the internal milieu. The strongest connections with limbic cortices are with the cingulate cortex. The rostral half of area 46 is connected with anterior cingulate area 32 (Barbas and Mesulam, 1985; Barbas, 1988; Barbas and Pandya, 1989), and the rostral part of area 24; the latter has weak connections with caudal area 46. Caudal area 24 is connected with almost the entire extent of area 46 (Baleydier and Mauguiere, 1980; Morecraft and Van Hoesen, 1993; Arikuni et al., 1994). The interaction of area 46 with the anterior cingulate, and adjacent areas 10 and 9, appears to be important in tasks with high cognitive demands [reviewed in (Paus, 2001; Taylor et al., 2007; Walton et al., 2007)]. The anterior and dorsal parts of area 46 have bidirectional connections with the posterior cingulate as well (area 23) (Baleydier and Mauguiere, 1980; Pandya et al., 1981; Morecraft et al., 2004). Some of the cingulate sites within areas 24 and 23 are part of the cingulate motor areas, as described below.

A significant source of connections of area 46 includes areas associated with the hippocampal system, though not the hippocampus proper (the subiculum and ammonic fields do not project to area 46). These cortical areas include the retrosplenial cortex (areas 29 and 30), as well as the presubiculum and parasubiculum, which project robustly to the caudal tip of dorsal area 46, the neighboring gyral cortex ventrally, and the mid-dorsal part of the principal sulcus (Barbas and Blatt, 1995; Parvizi et al., 2006; Kobayashi and Amaral, 2007), in pathways that are reciprocal (Selemon and Goldman-Rakic, 1988; Morris et al., 1999a). Area 30 projects to the rostral half of area 46 (Kobayashi and Amaral, 2007). These medial temporal areas have been referred to as the caudomedial lobule (Goldman-Rakic et al., 1984). The parahippocampal area TF sends moderate projections to the depths of the principal sulcus at the middle rostro-caudal extent of area 46 (Lavenex et al., 2002), in pathways that are reciprocal (Suzuki and Amaral, 1994), and issues sparse projections to caudal area 46 (Lavenex et al., 2002). The central part of area 46 sends moderate or sparse projections to parahippocampal areas TF and TH (Suzuki and Amaral, 1994). The relatively robust linkage of area 46 with retrosplenial cortices has been interpreted as evidence that these structures have a role in working memory (Morris et al., 1999b). An equally plausible explanation is that area 46 accesses information from areas involved in long-term memory in order to complete the task at hand, such as remembering where kitchen utensils are stored in the process of preparing a meal.

Area 46 has light and bidirectional connections with the amygdala (Aggleton et al., 1980; Ghashghaei et al., 2007), a structure associated with emotional processes. The projections from

the amygdala to the caudal part of area 46 originate in the dorsal part of the basolateral nucleus, which receives projections from visual association cortices [for discussion and review of relevant literature see (Barbas and De Olmos, 1990)].

Connections with premotor cortices

Area 46 is strategically positioned for action in cognitive operations through its robust connections with most premotor areas, including the dorsal and ventral premotor cortex, as well as rostral and ventral cingulate motor areas, the SMA and pre-SMA (Selemon and Goldman-Rakic, 1988; McGuire et al., 1991; Bates and Goldman-Rakic, 1993; Lu et al., 1994; Wang et al., 2001; Hatanaka et al., 2003; Tachibana et al., 2004; Takada et al., 2004). Projections from area 46 target specifically the forelimb representation within the rostral and caudal cingulate motor areas (Bates and Goldman-Rakic, 1993). Moreover, area 46 is interlinked with sites that represent the entire arm in the ventral premotor area (Lu et al., 1994). Ventral area 46, in particular, is the only prefrontal area that has substantial projections to the digit representation within the ventral premotor area (Dum and Strick, 2005). In addition, area 46 has bidirectional connections with the larynx area, which is also situated in ventral area 6 (Simonyan and Jurgens, 2005). The dorsal sector of area 46 projects to several cingulate motor areas, including area 23c and area 24c, but only its caudal sector projects sparsely to M2 (Morecraft and Van Hoesen, 1993).

Connections with the thalamus

The subsectors of area 46 have in common robust bidirectional connections with the mediodorsal thalamic nucleus, concentrated predominantly in its parvocellular division [e.g., (Giguere and Goldman-Rakic, 1988; Siwek and Pandya, 1991; Barbas et al., 1991; Erickson and Lewis, 2004)]. A minority of thalamic connections include the medial pulvinar, the ventral anterior, intralaminar and anterior nuclei (Gutierrez et al., 2000; Trojanowski and Jacobson, 1977; Barbas et al., 1991; Romanski et al., 1997; Xiao and Barbas, 2002; Xiao and Barbas, 2004; Xiao et al., 2009). Neurons in the mediodorsal thalamic nucleus, like those in area 46, fire when monkeys keep information on line during the delay period of the delayed response tasks (Alexander and Fuster, 1973; Fuster and Alexander, 1973), and their response is disrupted when cooling incapacitates area 46 (Alexander and Fuster, 1973). This evidence demonstrates the intricate relationship of the bidirectional corticothalamic pathways in a task that requires mnemonic processing on a temporary basis (Kubota et al., 1972; Alexander and Fuster, 1973; Kubota et al., 1980; Wilson et al., 1993).

Recent findings have shown that area 46, like all other cortices, projects to the thalamic reticular nucleus, which is entirely inhibitory, and is thought to gate information between the thalamus and the cortex [reviewed in (Barbas and Zikopoulos, 2007; Zikopoulos and Barbas, 2007a)]. However, unlike most other prefrontal cortices, which project only to the anterior sector of the reticular nucleus, dorsal area 46 issues more widespread projections, reaching reticular sites that receive projections from visual, auditory, somatosensory and polymodal cortices. Moreover, these central and caudal sectors of the thalamic reticular nucleus have bidirectional connections with thalamic nuclei that are connected with sensory and polymodal cortices (Zikopoulos and Barbas, 2006). This evidence suggests that area 46 may be in a unique position to gate sensory input through the thalamic reticular nucleus to select behaviorally relevant stimuli and suppress distracters at a very early stage of processing [reviewed in (Barbas and Zikopoulos, 2007; Zikopoulos and Barbas, 2007a)].

6. Neurotransmitter-specific projections to area 46 and age-related changes in neurotransmitters

As with other cortical areas, an important source of projections to area 46 is from neurotransmitter-specific systems in the basal forebrain and the brainstem. Dopaminergic fibers originating in the ventral tegmental area (Porrino and Goldman-Rakic, 1982; Williams and Goldman-Rakic, 1998), reach all layers of area 46 but are densest in the deep part of layer 1 and the adjacent layer 2, and in layers 5–6 (Lewis et al., 1988; Berger et al., 1988; Smiley and Goldman-Rakic, 1993). Noradrenergic fibers, which originate from the locus coeruleus (Porrino and Goldman-Rakic, 1982), have a different laminar distribution than the other modulator systems. Noradrenergic fibers innervate all layers of area 46 but are most densely distributed in the middle cortical layers, including layer 5, the deep part of layer 3 and layer 4 (Lewis and Morrison, 1989). Cholinergic projections (assessed by labeling with choline acetyltransferase, the biosynthetic enzyme, or acetylcholinesterase, the degradative enzyme for acetylcholine) originate from the basal forebrain, and innervate lightly most layers of area 46, and moderately layers 1, 2 and the upper part of layer 3 (Mesulam et al., 1984; Lewis, 1991; Mrzljak et al., 1995; Ghashghaei and Barbas, 2001). The serotonergic (5-HT) system, which originates in the raphe nuclei (Porrino and Goldman-Rakic, 1982), moderately innervates layers 1 and 2, and lightly all other layers of area 46 (Smiley and Goldman-Rakic, 1996).

The neurotransmitter-specific systems have complex and complementary roles in the cognitive functions of area 46. For example, working memory functions depend on optimal levels of DA, resembling an inverted U pattern, so that too little or too much DA impair working memory, whereas a level in between these extremes is optimal (Arnsten, 2006). The effects of DA on working memory appear to be mediated through D1 receptors (Sawaguchi and Goldman-Rakic, 1991), so that antagonists of D1 receptors, introduced in low levels by iontophoresis, improve memory field activity in area 46, whereas high doses inhibit neuronal firing (Williams and Goldman-Rakic, 1995).

The noradrenergic locus coeruleus shows varied levels of activity that correlate with behavioral states [(Usher et al., 1999); reviewed in (Aston-Jones et al., 1999; Aston-Jones and Cohen, 2005)]. Very low levels of activity are associated with poor performance on a discrimination task in monkeys. At higher levels, performance is optimal, and at very high levels performance decreases, resembling a classic inverted U function (Aston-Jones et al., 1999). In the monkey dorsolateral prefrontal cortex, specifically in area 46, norepinephrine has differential effects depending on the type of receptor activated. Activation of $\alpha 2A$ receptors enhances working memory, while activation of $\alpha 1$ and $\beta 1$ receptors impairs working memory (Arnsten, 2001; Arnsten, 2006). Disturbance in the balance of the level of dopamine and norepinephrine may help explain the distractibility that occurs in attention deficit hyperactivity disorder (Arnsten and Li, 2005).

The neurotransmitter-specific systems are differentially affected in normally aging monkeys. Monkeys between 10 and 18 years of age exhibit a 50% drop in dopamine levels in area 46, shown by biochemical measurement of dopamine and its metabolite HVA (Goldman-Rakic and Brown, 1981; Wenk et al., 1989). By contrast, D1 receptor binding is unaltered with age in the monkey area 46 (Moore et al., 2005). There is little or no reduction in norepinephrine or serotonin during normal aging in monkeys (Goldman-Rakic and Brown, 1981), however, receptor-binding studies have revealed a significant reduction in both $\alpha 1$ and $\alpha 2$ norepinephrine receptor binding with age in the superficial layers of area 46 (Moore et al., 2005). Further, administration of noradrenergic $\alpha 2$ receptor agonists to aged monkeys improves their performance on the Delayed Response task (Arnsten and Contant, 1992; Berridge et al., 1993). Recently it has also been demonstrated that a $\beta 2$ agonist enhances

working memory in aged monkeys (Ramos et al., 2008). Interestingly, monoamine receptor binding has a significant negative linear relationship with performance on DNMS, DRST and CSST (Moore et al., 2005). There is also evidence that serotonin and serotonin receptors are down regulated with age: in particular 5HT, 5HT1 and 5HT2 receptors are reported to be reduced in area 46 in aged monkeys (Goldman-Rakic and Brown, 1981; Wenk et al., 1989; Bigham and Lidow, 1995).

The cholinergic system plays an important role in learning and memory and has been the focus of numerous studies, but there is a lack of clarity and consistency in data on the effects of aging on this neurotransmitter system. For example, one study reported a modest reduction in ChAT levels in the frontal pole of aged rhesus monkeys (Wenk et al., 1989), but a subsequent study reported that the levels of this synthetic enzyme were not changed in the frontal cortex in a different cohort of aged monkeys (Wenk et al., 1991). Further, another study reported that neither M1 nor nicotinic ACh receptors are reduced with age in monkey prefrontal cortex (Wenk et al., 1989) while a subsequent study reported a significant decrease in M1 receptor binding in area 46 (Vannucchi and Goldman-Rakic, 1991). Clearly further work is required to unequivocally demonstrate whether cholinergic systems are substantially changed with age in the primate area 46.

Finally, there is evidence that both glutamatergic and GABAergic transmitter systems are altered with age in the neocortex. With regard to the glutamatergic system, this evidence includes findings of dendritic regression and loss of dendritic spines, the principal postsynaptic substrates for glutamate action (Jacobs et al., 1997; Page et al., 2002; Duan et al., 2003), a decrease in the number of neurons expressing glutamatergic GluR2 and NMDAR1 receptor subunits (Hof et al., 2002), and a significant decrease in the number of asymmetric synapses in area 46 (Peters et al., 2008a). Finally, there is a significant increase in binding density for the benzodiazepine receptor modulatory site on the GABA_A receptor in the neocortex of aged monkeys, without a change in the binding density of GABA_A receptors (Rosene and Nicholson, 1999).

7. Age-related alterations in the morphology of neurons

In Nissl stained sections of both monkey and human cortices it can be seen that aging neurons acquire some lipofuscin in their cell bodies, but in this type of preparation there are no other obvious alterations of neurons. However, when Golgi preparations, neuronal tracers, or intracellular filling are employed, a different picture emerges.

Some of the most comprehensive studies of the effects of age on neurons have been carried out by injecting tracers into area 46 and then examining the labeled cortico-cortical projection neurons in the temporal cortex [e.g., (Page et al., 2002; Duan et al., 2003)]. These studies have shown that when young and old monkeys are compared, there are only minor qualitative differences in the dendritic lengths and complexity of dendritic trees of the pyramidal neurons, but there is extensive loss of dendritic spines from all portions of the dendritic tree. Overall, there is a loss of some 25% of dendritic spines on apical and basal dendrites (Duan et al., 2003). A similar observation was made in a later study that examined the effects of normal aging on the structural properties of layer 2/3 pyramidal neurons in area 46 from rhesus monkeys (Kabaso et al., 2007). In addition to reduced branch lengths in the apical dendrites and increased numbers of segments in the basal dendrites, the aged neurons had reduced numbers of dendritic spines, amounting to 50%, with fewer thin and more stubby spines being evident (Kabaso et al., 2007). Since thin and small spines are important for learning, the shift from thin to stubby spines may contribute to the learning deficits that occur during aging. In a recent study Kabaso et al. (2009) have shown that not all pyramidal neurons in the cortex are affected in the same way by age. Thus Kabaso et al. (2009) find that although all pyramidal

cells lose spines, the dendrites of the long projecting neurons from superior temporal cortex to area 46 become shorter and less branched, while the lengths of dendrites of locally projecting pyramidal cells in area 46 are largely unaltered.

These recent studies on monkey cortex largely confirm earlier work on the effects of age on neurons in monkey prefrontal cortex (Cupp and Uemura, 1980). Examination of Golgi impregnated neurons revealed that with age entire branches are lost from the apical dendrites of pyramidal neurons, accompanied by a loss of about 25% of dendritic spines from all branches of the dendritic trees. By contrast, another study of Golgi impregnated neurons from prefrontal area 10 and visual area 18 of young and old human cerebral cortices concluded that with age there is only a slight loss in the overall extent of the dendritic trees of pyramidal neurons but a dramatic loss of about 50% of dendritic spines (Jacobs et al., 1997).

The loss of spines on area 46 pyramidal neurons during normal aging in the monkey is paralleled by a loss of synapses. Electron microscopic analyses of the numerical density of both asymmetric (excitatory) and symmetric (inhibitory) synapses in the neuropil of layer 2/3 and layer 5 of behaviorally tested rhesus monkeys reveal that with age there is no change in the length of the synaptic junctions, or in the percentage distribution of synapses relative to postsynaptic spines and dendritic shafts (Peters et al., 2008a). However, in layer 2/3 there is a loss of about 30% of synapses with age and both asymmetric and symmetric synapses are lost at the same rate. Layer 5 is different, since only about 20% of synapses are lost with age, and this is almost entirely due to a loss of asymmetric (excitatory) synapses. As far as we are aware, the only other study of the effects of aging on synapses in monkey prefrontal cortex is that of Uemura (Uemura, 1980), who used electron microscopy to examine superior frontal cortex, or area 9, which is situated above area 46 and has related functions. The overall loss of synapses calculated in this study (Uemura, 1980) is similar to that of Peters et al. (Peters et al., 2008a), who also found loss of synapses from the upper and middle thirds of the depth of the cortex to be greater (25%) than from the lower third (16%).

A piecewise analysis of the data brought to light the interesting fact that most of the synapse loss occurs after 20 years of age. When the synapse loss data are correlated with the cognitive status of the monkeys, it emerges that for layer 2/3 there is a strong correlation between cognitive impairment and the numerical density of asymmetric synapses and a weaker correlation between symmetric synapse loss and cognitive impairment. In contrast, for layer 5 there is no correlation between synapse loss and cognitive impairment.

The greatest loss of synapses (30–60%) is found in layer 1, which becomes significantly thinner during aging (Peters et al., 1998b). The loss of synapses in layer 1 is accompanied by a reduction in the frequency of profiles of dendrites and dendritic spines. In layer 1 there is a significant correlation between the numerical density of synapses and the cognitive decline exhibited by aged monkeys. Interestingly, although there is a similar thinning of layer 1 in area 17 of the monkey cortex, accompanied by a loss of dendritic branches, dendritic spines and synapses, these alterations do not correlate with behavioral changes in memory function. The probable basis for this difference is that while area 46 is implicated in cognition, the same is unlikely to be true of area 17 (Peters et al., 2001a).

The extensive loss of synapses from layer 1 of area 46 with age is very interesting in light of the fact that there is a massive input of thalamic afferents from the M (matrix) neurons in thalamic nuclei to layer 1 of cerebral cortex in several species [reviewed in (Jones, 2007)]. Moreover, inputs from several thalamic nuclei in rats converge on layer 1 throughout the cerebral cortex (Rubio-Garrido et al., 2009). These findings are consistent with the strong projections from most thalamic nuclei to layer 1 in monkeys, which run in parallel to the classically described thalamic projection system to the middle layers of the cortex [reviewed

in (Jones, 1998; Jones, 2007)]. Recent studies show that in rhesus monkeys there is a significant projection from the ventral anterior thalamic nucleus to the entire prefrontal cortex, including area 46 (Zikopoulos and Barbas, 2007b). In addition to projections to the middle layers, significant projections from the ventral anterior thalamic nucleus reach layer 1 of area 46 and the adjacent area 9 (Zikopoulos and Barbas, 2007b). The large majority of these projections originate from calbindin positive 'matrix' thalamic neurons. It is highly probable that there is a similar input to layer 1 of area 46 by other thalamic nuclei in the monkey. The reduction in the number of dendritic branches and synapses from the apical tufts would interfere with the feedback interactions between area 46 and thalamic nuclei, which appear to be crucial for associative learning and attention.

The results of electron microscopic analyses of aging and synapse numbers in human frontal cortex are much more confusing. For example, Scheff et al. (Scheff et al., 2001) examined layers 3 and 5 in area 9 from the brains of cognitively normal humans and concluded that during the first eight decades of life there is no significant loss of synapses. These findings support a similar conclusion reached earlier by another group, who examined synapses in layer 3 of frontal cortex (Huttenlocher, 1979). In contrast, another study reported a significant loss of synapses with age from human frontal, but not temporal cortex, in brains from "apparently intellectually normal people". Interestingly, when these brains were compared with ones from patients diagnosed as suffering from Alzheimer type dementia, the numbers of synapses in the two groups were similar (Gibson, 1983). The results of determining synapse numbers in the frontal cortices of human brains using antibodies, such as ones against synaptophysin, to label axon terminals are also conflicting. For example, several studies found that individuals older than 60 years showed an average of 20% decrease in the numbers of synapses in the frontal cortex compared to young individuals (Masliah et al., 1993). By contrast, a fourth study found no loss of synapses (Zhan et al., 1993).

Since there is a loss of dendritic spines from pyramidal neurons in non-human primate frontal cortex with aging, it follows that there has to be a loss of synapses involving these spines. Why disagreement has arisen about whether there is a loss of synapses from human frontal cortex with normal aging is not clear, but various authors have attributed the discrepant findings to factors such as the inadvertent inclusion of patients with early stages of Alzheimer's disease among the normal subjects, morphological changes brought about by long postmortem intervals, and inappropriate methods used to make accurate synaptic counts.

8. Age-related changes in nerve fibers and in myelin

MRI studies of both monkeys [e.g., (Lai et al., 1995b)] and humans [e.g., (Albert, 1993; Guttmann et al., 1998)] have shown a loss of white matter from the cerebral hemispheres with age, and especially from the frontal lobes. To determine how much of a loss of nerve fibers from monkey white matter occurs with age, several fiber tracts have been examined by electron microscopy. These tracts include the anterior commissure (Sandell and Peters, 2003), the optic nerve (Sandell and Peters, 2001), as well as the fornix, the splenium of the corpus callosum and the anterior cingulate bundle (unpublished data). In each of these tracts there is a significant loss of myelinated nerve fibers (20 to 40%) with age. Any loss of white matter must result in some disconnection between various components of the central nervous system. Of the tracts for which there is information on both the extent of nerve fiber loss and the cognitive status of the monkeys, there are significant correlations between the two measures for the fornix, and anterior commissure, but not for the splenium of the corpus callosum or for the cingulate bundle. However, for the cingulate bundle there is a significant correlation between cognition and the frequency of myelinated nerve fiber profiles and degenerating axons (M. Bowley: personal communication). This is of interest since the cingulate bundle is a heterogeneous tract

that connects the prefrontal cortex, the thalamus, striatum, cingulate gyrus, parietal cortex and medial temporal lobe (Mufson and Pandya, 1984; Schmammann and Pandya, 2006).

Although there is substantial loss of myelinated nerve fibers from white matter in aging primates [e.g., (Peters et al., 2000; Peters, 2007)], only few appear to be lost from cortical gray matter. However, there are age-related changes in the structure of myelin sheaths in both white and gray matter of the cerebral hemispheres [e.g., (Feldman and Peters, 1998; Peters et al., 2000; Peters and Sethares, 2002a)], since some nerve fibers show degenerative alterations (Fig. 3). These alterations include focal splitting of the sheaths to accommodate dense cytoplasm of the oligodendrocytes that form the sheaths, and ballooning of sheaths by accumulation of fluid in splits of myelin sheaths (Fig. 3). There is reason to believe that these degenerative changes are due to the degeneration and death of oligodendrocytes.

Other age-related myelin alterations are considered to be regenerative. One is the thickening of sheaths by addition of myelin lamellae, especially to the sheaths of larger diameter fibers (Peters et al., 2001b), and another is the formation of sheaths that contain redundant myelin, so that the loose sheaths are much too large for the size of the ensheathed axons.

When the frequency of occurrence of these age-related alterations in myelin are assessed in area 46 of rhesus monkeys, it is found that they increase significantly in frequency with age. The frequency of occurrence of profiles of sheaths that show myelin alterations also correlates significantly with the cognitive impairment shown by the monkeys (Peters and Sethares, 2002a). It is likely that myelin defects slow down the rate of conduction along the nerve fibers, so that the timing in neuronal circuits is affected.

Another alteration in myelin sheaths in area 46 is a 90% age-related increase in the frequency of occurrence of profiles of paranodes, indicating an increase in the numbers of internodal lengths of myelin with age (Peters and Sethares, 2003). This increase would occur if some of the original lengths of myelin degenerate and are replaced by shorter internodes with thinner myelin sheaths. Indeed, shorter internodal lengths of myelin and abnormally thin myelin sheaths have been found in the cortices of old monkeys (Peters and Sethares, 2003). The increase in paranodal profiles in area 46 correlates significantly with both age and cognitive impairment, and it has been suggested that the correlation with impairment occurs because of an increase in the number of internodes, and hence the number of nodes of Ranvier along nerve fibers, which slows conduction speed. This conclusion fits with earlier reports of a reduced conduction velocity along myelinated nerve fibers in the spinal cord of old compared to young cats (Morales et al., 1987). More directly, the slowing of conduction velocity by the interposition of short internodal lengths on conduction velocity has also been demonstrated directly in the rubrospinal tract of the mouse as it remyelinated following a crush lesion (Lasiene et al., 2008). It should also be pointed out that with aging there is some reorganization of nodes of Ranvier in monkey central nervous system (Hinman et al., 2006). As some sheaths become thicker with age, the paranodal loops become disorganized, so that not all of them reach the axon to form membranous junctions. There are also alterations in the molecular organization of this region, because with age the voltage-dependent potassium channels normally present in the juxtaparanodal region become mislocalized to the paranodal regions, and this may be detrimental to the maintenance of axonal conduction. No comparable studies are available for human brains, since obtaining human material that is well enough preserved to carry out such studies is extremely difficult.

9. Age-related changes in neuroglial cells

With increasing age, astrocytes, oligodendrocytes and microglial cells accumulate some inclusions in their perikarya. This is especially true of the astrocytes, which in normal aging appear to be the primary phagocytes in the cerebral cortices of primates. What material is being

phagocytosed by astrocytes has not been fully determined, but it is clear that they do phagocytose degenerating myelin sheaths, since pieces of myelin sheaths are sometimes evident within astrocytes, and some of the more amorphous inclusions can be labeled with antibodies to myelin basic protein (Peters and Sethares, 2003). Although astrocytes become more fibrous with age, there is no evidence that they hypertrophy, other than in layer 1. The glial limiting membrane becomes thicker in layer 1 and astrocytes enlarge to fill the spaces in the neuropil produced by degeneration of portions of the apical dendritic trees of pyramidal neurons. Astrocytes also become more filamentous (Bandler et al., 1991). However, there is no increase in the number of astrocytes in layer 1 of area 46 with age (Peters and Sethares, 2002b). Microglia also contain inclusions in aging monkeys, but surprisingly they do not normally seem to be involved in phagocytosing degenerating myelin, since there is no convincing evidence that any of the inclusions in microglia are labeled with antibodies to myelin basic protein (Peters and Sethares, 2003); the same is true of the inclusions in oligodendrocytes.

Recently, we have examined the effects of age on the populations of neuroglial cells in primary visual cortex (Peters and Sethares, 2004; Peters et al., 2008b) and in area 46 (unpublished data). While there are no changes with age in the numbers of astrocytes and microglial cells, there is a 45 to 50% increase in the numbers of oligodendrocytes. Oligodendrocyte number does not correlate with cognitive impairment, but it does correlate with the age-related increase in the numbers of profiles of paranodes of myelinated nerve fibers. This evidence suggests that increased numbers of oligodendrocytes are required to produce the shorter internodal lengths that are associated with remyelination.

As pointed out in the previous section, there is strong evidence that some myelin internodes degenerate with age, probably due to the degeneration of their parent oligodendrocytes, which may be reflected by the formation of bulbous enlargements along the processes of some oligodendrocytes in older monkeys. These processes are filled with characteristic inclusions [see (Peters et al., 1991; Peters, 1996)], which are akin to those found within the cell bodies of oligodendrocytes in the cerebral cortices of older monkeys. However, it has yet to be established that these inclusions are a sign of oligodendrocyte degeneration.

There is no information available for primates, but in adult rodents some oligodendrocytes normally die with age while others are generated and it is the imbalance between these two processes that leads to the increase in the numbers of oligodendrocytes with age (Cerghet et al., 2006). It is possible that newly generated oligodendrocytes are derived by division of existing oligodendrocytes, which would explain the presence of groups of oligodendrocytes in the aging cerebral cortex. However it is much more likely that new oligodendrocytes are derived from the oligodendroglial progenitor cells. These progenitor cells are recognized by the fact that they label with antibodies to the platelet-derived growth factor α receptor PDGFRA/NG2. Such cells are distributed throughout the adult central nervous system and it has recently been shown that in adult mice these precursor cells generate oligodendrocytes that are capable of myelinating axons (Rivers et al., 2008). It is likely that this is also true in the monkey, since NG2 labeled cells have been identified in monkey cerebral cortex [see (Peters, 2004; Peters and Sethares, 2004)]. Interestingly, when such precursor cells are examined in the electron microscope they closely resemble protoplasmic astrocytes, which may help explain why they were overlooked in early studies of the fine structure of cerebral cortex.

10. Age-related changes in the electrophysiological properties of layer 3 and layer 5 pyramidal neurons in area 46 of the rhesus monkey

As discussed above, there is abundant evidence that individual pyramidal neurons in area 46 of the rhesus monkey undergo significant structural changes during normal aging. It is well

established by experimental and theoretical modeling studies that the structural properties of a given neuron play a critical role (together with connectional and intrinsic ion channel properties) in determining its electrophysiological properties. Specifically, dendritic and somatic structural properties determine the passive electrotonic properties of a given neuron. In turn, the electrotonic properties are fundamental determinants of synaptic integration and neural firing patterns (Mainen and Sejnowski, 1996; Euler and Denk, 2001; Vetter et al., 2001; Krichmar et al., 2002; Weaver and Wearne, 2008). Thus, the significant age-related changes in neuronal structure described above - dendritic regression, loss of dendritic spines and synapses, and myelin dystrophy, in particular - would be expected to have a major impact on the functional properties of individual neurons. For example, one might plausibly predict that dendritic regression would lead to increased input resistance with concomitant increases in excitability, or that significant loss of dendritic spines would lead to a decrease in the frequency and amplitude of glutamatergic excitatory synaptic responses. Indeed, most studies have hypothesized that the significant structural alterations to neurons that occur with age are likely to have significant and detrimental functional consequences. In view of the mounting evidence of structural changes in neurons in primates, the dearth of studies on the effects of aging on the electrophysiological properties of neurons is striking. To our knowledge, to date only two *in vivo* studies have examined the effects of aging on the electrophysiological properties of neocortical neurons in the monkey. In these, Leventhal and coworkers demonstrated that visual cortical neurons in aged monkeys exhibit significantly increased spontaneous action potential (AP) firing rates, and that these alterations are associated with degradation of visual stimulus selectivity (Schmolesky et al., 2000; Leventhal et al., 2003). These tantalizing findings highlight the need for similar studies to be performed in the prefrontal cortex of cognitively characterized young, middle-aged and old rhesus monkeys which are an excellent model for humans, in which neither *in vivo* nor *in vitro* electrophysiological experiments such as those described here are possible.

Over the past several years we have begun to examine the effects of age on neuronal function in the non-human primate by recording from pyramidal neurons in *in vitro* slices prepared from area 46 of behaviorally characterized young and aged rhesus monkeys. In this series of studies, whole cell patch clamp recordings have been used to assess the basic electrophysiological properties of both layer 3 (primarily cortico-cortical) and layer 5 (primarily cortico-striatal) pyramidal neurons (Luebke et al., 2004; Chang et al., 2005; Chang and Luebke, 2006; Luebke and Chang, 2007). As a result, we have demonstrated that fundamental electrophysiological properties of layer 3, but not layer 5, pyramidal neurons are significantly changed with age, and that these changes are associated with cognitive performance within the aged cohort of monkeys from which slices were prepared.

Passive membrane properties

The passive membrane properties of a neuron are its resting membrane potential, membrane time constant and input resistance. These passive properties (determined by the structural membrane characteristics) play a determinative role in the entire repertoire of electrophysiological features of a given neuron, including AP firing rates and patterns, presynaptic activity and postsynaptic responses. As an example, neurons having high input resistance will, under normal conditions, respond to a given depolarizing input with a larger membrane potential change (greater depolarization, or increased excitability) than a cell with low input resistance. Because passive properties are integral to the electrophysiological phenotype of all neurons, determination of the effects of normal aging on these properties is of paramount importance, particularly in light of the known age-related changes in the structure of area 46 pyramidal neurons in the monkey. The resting membrane potential and time constant of both layer 3 and layer 5 pyramidal neurons in area 46 are unaltered with age, while the input resistance of layer 3, but not layer 5, pyramidal neurons is significantly increased (Chang et

al., 2005; Luebke and Chang, 2007). The basis for this increase in input resistance of layer 3 pyramidal neurons remains to be determined, but, as described below, the increase is associated with a significant, functionally important increase in the excitability of these neurons in *in vitro* slices of area 46.

Repetitive AP firing properties

The most functionally relevant age-related alteration in electrophysiological properties observed in area 46 of the monkey to date is a dramatic increase in the frequency of AP firing of layer 3, but not layer 5, pyramidal neurons (Chang et al., 2005; Luebke and Chang, 2007). For example, the mean frequency of APs elicited in layer 3 pyramidal neurons by a 2 second 180 pA depolarizing current step is almost two times greater in old, as compared to young monkeys. Interestingly, there is a significant positive linear relationship between firing rate and input resistance, indicating that increased input resistance is a significant contributor to increased firing rate. It is possible that age-related alterations in the ionic currents responsible for the timing of spike trains play an important role in the observed increase in excitability of aged layer 3 pyramidal neurons. An increase in excitatory synaptic inputs, or a decrease in inhibitory synaptic inputs could also plausibly contribute to increased firing rates. However, the evidence (discussed below) for significantly decreased excitatory and increased inhibitory synaptic responses in layer 3 pyramidal neurons of prefrontal cortex from aged monkeys (Luebke et al., 2004) is not consistent with this idea.

Excitatory and inhibitory synaptic response properties of layer 3 pyramidal neurons in area 46 of aged monkeys

Normal aging results in a dramatic loss of dendritic spines and of synapses in layer 3 pyramidal neurons of the prefrontal cortex (discussed above). The significant loss of this postsynaptic substrate suggests that there will be significant changes in the glutamatergic synaptic response properties of these neurons. Indeed, whole cell patch clamp recordings have demonstrated that spontaneous excitatory postsynaptic currents (sEPSCs) in layer 3 neurons are significantly reduced in frequency with age (Luebke et al., 2004). These changes could be due to a loss of dendritic spines and synapses, or to a reduction in the release of glutamate from presynaptic terminals. Given the clear evidence for increased, not reduced, excitability of layer 3 pyramidal neurons, which provide a majority of the glutamatergic input to each other in normal aging, we favor the first idea (Chang et al., 2005). On the other hand, the amplitude and kinetics of miniature excitatory PSCs are not significantly altered with age. This evidence suggests that AMPA receptors present on the remaining spines are not significantly altered with age. In marked contrast to EPSCs, the frequency of GABA_A receptor-mediated spontaneous inhibitory PSCs are significantly increased with age in layer 3 pyramidal neurons (Luebke et al., 2004). This finding is somewhat paradoxical in view of the finding that symmetric synapses are reduced in this brain area with age (Peters et al., 2008a). Perhaps the most straightforward explanation for the increase in spontaneous inhibitory PSC frequency is an increase in the action potential-dependent release of GABA from presynaptic interneurons; however this hypothesis has yet to be directly tested. As with excitatory synaptic events, the frequency, amplitude, and kinetics of *miniature* inhibitory PSCs are not significantly different in neurons in young and old monkeys, indicating that the properties of the GABA_A receptor *per se* and the GABA release probability are not markedly altered with age in these cells.

Implications of age-related electrophysiological changes for age-related decline in cognitive functions mediated by the prefrontal cortex in non-human primates

The series of electrophysiological studies described here have yielded several findings of relevance to an understanding of the neural basis of age-related decline in executive function mediated by area 46 in monkeys. In a broad sense, one of the most interesting findings is the

significant change in the electrophysiological properties of layer 3 pyramidal neurons with age, while layer 5 pyramidal neurons are relatively spared. This is interesting in light of the fact that area 46 projects to other cortices primarily from layer 3 (Barbas and Rempel-Clower, 1997), and layer 3 neurons are thought to play a more central role in the cognitive abilities mediated by area 46 than layer 5 neurons, which project largely to the striatum. The second key finding is that firing rates of layer 3 pyramidal neurons are significantly changed in slices prepared from aged monkeys, and that these rates are related, in a U-shaped manner, to the cognitive performance of the monkeys from which the slices were prepared. Across all behavioral tasks, AP firing rates assessed *in vitro* are significantly related to the performance of the aged monkeys, such that the cells with the lowest and highest firing rates were from slices prepared from monkeys that exhibited poor performance while those with intermediate firing rates were from slices prepared from monkeys that exhibited good performance. This suggests that performance is dependent on a precise pattern of neural activity, such that firing rates that are too low are insufficient to differentiate relevant signals from background noise, and firing rates that are too high result in increased “noise.” Such U-shaped relationships are not unusual in biological systems. These findings are consistent with the idea that there is an optimal firing rate in aged monkeys that has a higher frequency than in young monkeys. Such a shift may plausibly be a compensatory response to increased AP conduction failure (Rosene et al., 2003) secondary to the extensive myelin dystrophy seen in the primate prefrontal cortex with age (reviewed in (Peters, 2002)). Thus in the aged prefrontal cortex, higher rates of firing may be required to maintain functions that are encoded by lower rates in the young prefrontal cortex.

11. Conclusions

Table 1 and Figure 4 summarize what is currently known about the effects of age on area 46 in monkeys. Senile plaques, although few in number in the rhesus monkey cortex, do become more frequent with age, but the increase has no correlation with cognitive decline. Other parameters, such as volume of the gray matter and the number of neurons per unit volume, do not appear to change significantly to any extent, and so these factors are also unlikely to be the basis of cognitive decline. However, analysis of digitized images suggests that the arrangement of neurons into microcolumns becomes somewhat disorganized with increasing age, and this does correlate with cognitive decline, although the reason is not known. Whether there is an age-related decrease in the overall thickness of area 46 is still debatable. However, there is thinning of layer 1 with age. This is associated with loss of dendrites and synapses from the apical tufts of pyramidal neurons, which correlate with the DNMS behavioral test with a 2 minute delay. Layer 1 has a significant role in the function of cortical columns, as recipient of the dendrites from pyramidal neurons in the cortical layers below [reviewed in (Vogt, 1991)]. In addition, layer 1 is enriched with inputs from external sources, and receives significant projections from subcortical neurotransmitter-specific systems, from the matrix (calbindin-specific) system of the thalamus, and from cortico-cortical feedback projections.

Other than the effects on their apical tufts with age, pyramidal neurons in area 46 lose some of their other dendrites, and some dendrites are shortened. Importantly, as many as 25 to 50% of dendritic spines are lost with age, paralleled by an overall loss of both excitatory and inhibitory synapses from the neuropil. In layer 2/3 the loss of as many as 30% of synapses with age correlates significantly with cognitive decline. However, the biggest loss of synapses is in layer 1 (30 to 60%), affecting mostly the apical dendritic tufts of pyramidal neurons. As is the case with layers 2/3, there is a correlation between synapse loss from layer 1 and cognitive decline. In contrast to the upper layers of the cortex, loss of synapses from layer 5 is only about 20% and does not correlate with cognitive decline. The origins of the axon terminals that formed these lost synapses are not known, since they could be from either intrinsic or extrinsic sources. However since the intrinsic excitatory neurons provide at least 90% of the axospinous

synapses in the cortex it is likely that they are the primary source of axospinous synapses that are lost with age. The loss of inhibitory synapses must be from the axonal plexuses of intrinsic inhibitory neurons, since the available evidence shows that inhibitory input in primates arises overwhelmingly from intrinsic sources at the level of cortical columns (Melchitzky et al., 2001).

Since there is a ubiquitous loss of white matter with age, and white matter contains the extrinsic myelinated nerve fibers that interconnect areas of the cortex with each other, it is highly likely that the extrinsic inputs to area 46 are reduced with age. It would follow that with age area 46 becomes partially disconnected. As for transmitters, it is known that there is a decrease in dopamine levels in area 46 with age, and a decrease in norepinephrine, serotonin and acetylcholine receptors. Further studies are needed to examine the effects of these age-related decreases in transmitter and receptor levels on cognition, since altering the levels of these extrinsic transmitters can affect behavioral performance.

With increasing age, many of the sheaths of the myelinated nerve fibers in both white and gray matter degenerate, so that many of the nerve fibers lose their original myelin sheaths and become remyelinated by shorter internodes with thinner sheaths. Both the increase in number of degenerating myelin sheaths and paranodes, and hence the increase in the number of internodal lengths of myelin correlate with cognitive decline. It is suggested that both the degeneration of sheaths and the formation of shorter internodes leads to a reduction in conduction velocity, so that the timing in neuronal circuits is affected, leading to cognitive impairment.

What is becoming clear from studies of area 46 and other cortical areas is that there is no single factor responsible for the cognitive decline associated with aging. For example, loss of nerve fibers from white matter results in some disconnections between parts of the central nervous system, reductions in the synaptic inputs to neurons alter the intrinsic physiological properties of neurons, and alterations in myelin sheaths result in reductions in conduction velocity so that the timing of neuronal circuits is affected (Fig. 4). At present we have only a general map to indicate the direction in which further studies should go. We hope that future research will fill in some of the details and lead to effective interventions in retarding the effects of cognitive decline which affects our increasingly aging population.

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Figure 1.

A, Lateral view of the rhesus monkey brain shows the extent of area 46 (small dotted line). Large dashed line is a diagrammatic representation of the upper and lower banks of the principal sulcus. Area 46 is divided into a rostral and a caudal sector (vertical dotted line). The thick vertical line shows the level at which the section shown in **B** was taken. **B**, Nissl stained coronal section through the frontal lobe at the level of the rostral end of the corpus callosum (cc). The principal sulcus (ps), which is on the lateral surface of the cortex, contains area 46 that extends over the upper and lower lips of the sulcus. **C**, Nissl stained section of area 46 in the lower bank of the principal sulcus shows the appearance of the six cellular layers and the underlying white matter (WM).

Figure 2.

The principal cortical connections of area 46. **A**, Lateral view of the rhesus monkey brain shows projections from sensory association areas originating from: visual (red); auditory (yellow); and somatosensory (green) association cortices; Projections are also shown for a visuomotor region in the lateral bank of the intraparietal sulcus (pink). The diagram shows the origin of projections and their termination in area 46, but connections with these cortices are reciprocal. **B**, Interconnections of area 46 within the prefrontal cortex. **C**, Medial (top), lateral (center) and ventral (bottom) views of the rhesus monkey brain show projections from limbic (shades of blue) and premotor cortices (brown). Abbreviations: CC, corpus callosum; names of sulci (shown with black letters): A, arcuate; C, central; Cg, cingulate; IP, intraparietal; L, lunate; LF, lateral fissure; P, principal; Rh, rhinal; ST, superior temporal. Silhouette numbers and letters show architectonic areas: Pre-SMA, pre-supplementary motor area; SMA, supplementary motor area. TF, area TF in anterior medial temporal lobe; TH in posterior medial temporal lobe (parahippocampal cortex).

Figure 3.

Electron micrographs show myelinated nerve fibers in area 46. **A** is from area 46 of a 10 year old monkey and shows that at this age the myelin sheaths around axons are compact. **B** is from area 46 of a 27 year old monkey, in which many of the myelin sheaths (**D**) have begun to degenerate and show dense inclusions within their lamellae. Other sheaths (asterisk) are empty because the axon has degenerated. Scale bars equal one micron.

Figure 4.

Summary of key age-related changes in area 46. Shown at top is a cartoon demonstrating the significant loss of dendritic spines and myelin dystrophy that occurs in pyramidal cells in area 46 with normal aging. Below this cartoon are representative data showing loss of dendritic spines, reduced frequency of glutamatergic synaptic currents, myelin dystrophy and increased action potential firing rates.

Table 1

Summary of variables that are known to change with age in Area 46 of the rhesus monkey. DNMs: delayed non-making-to-sample task; DRST: delayed recognition span task; CII: Cognitive Impairment Index.

	Direction of Change	Task	Correlation <i>P</i>	Citation
Variables that are known to be unchanged with age in A46				
Number of Neurons	na	na	na	Smith et al. 2004
Volume(assessed with neuroanatomical methods)	na	na	na	O'Donnell et al. 1999
Layer 5 pyramidal Cell-action potential frequency	na	na	na	Luebke & Chang, 2007
Layer 5 pyramidal Cell-slow AHP amplitude	na	na	na	Luebke et al.,(submitted)
Variables that change with age in A46 not tested for correlation with behavior				
Layer 3 pyramidal Cell-density of dendritic spines	Decreased	na	na	Duan et al, 2003
Layer 3 pyramidal Cell-frequency of glutamatergic EPSCs	Decreased	na	na	Luebke et al. 2004
Layer 3 pyramidal Cell-frequency of GABAergic IPSCs	Increased	na	na	Luebke et al. 2004
Dopamine levels	Decreased	na	na	Goldman-Rakic and Brown, 1981 Wenk et al., 1989
NE receptors	Decreased	na	na	Moore et al.,2005
5-HT receptors	Decreased	na	na	Goldman-Rakic, and Brown, 1981 Wenk et al., 1989; Bigham, and Lidow, 1995
Chat, MI receptors	?/Decreased	na	na	Vannucchi, and Goldman-Rakic, 1991
Variables that change with age in A46 and do NOT correlate with behavior				
Number of senile plaques	Increased	CII	na	Sloane et al.,1997
Number of oligodendrocytes	Increased	CII	na	Peters et al. (unpublished)
Number of synapses in Layer 5/6	Decreased	CII	na	Peters et al.,2008
Variables that change with age in A46 and correlate with behavior				
Gray matter volumes (assessed with MBI)	Decreased	DRST <i>best</i> DNMS	p<0.008 na	Alexander et al.,2008
“Strength” of microcolumns	Decreased	CII	p<0.01	Cruz et al.,2004
Thickness of Layer 1	Decreased	DNMS(2')	p<0.03	Peters et al., 1998
Number of synapses (total) in Layer 1	Decreased	CII	p<0.05	Peters et al.,1998

	Direction of Change	Task	Correlation <i>P</i>	Citation
Number of asymmetric synapses in Layer 2/3	Decreased	CII	p<0.008	Peters et al.,2008
Number of symmetric synapses in Layer 2/3	Decreased	CII	p<0.08	Peters et al.,2008
Number of abnormal myelin sheaths	Increased	CII	p<0.001	Peters et al.,2002
Number of paranodes	Increased	CII	p<0.01	Peters and Sethares,2003
Layer 2/3 pyramidal cell-slow AHP amplitude	Increased	CII,DNMS,DRST	p<0.03	Luebke et al.,(submitted)
Layer 2/3 pyramidal cell-action potential frequency	Increased	CII, DRST, DNMS	p<0.02	chang et al, 2005