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Age sensitivity of behavioral tests and brain substrates of normal aging in mice

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Knowledge of age sensitivity, the capacity of a behavioral test to reliably detect age-related changes, has utility in the design of experiments to elucidate processes of normal aging. We review the application of these tests in studies of normal aging and compare and contrast the age sensitivity of the Barnes maze, eyeblink classical conditioning, fear conditioning, Morris water maze, and rotorod. These tests have all been implemented to assess normal age-related changes in learning and memory in rodents, which generalize in many cases to age-related changes in learning and memory in all mammals, including humans. Behavioral assessments are a valuable means to measure functional outcomes of neuroscientific studies of aging. Highlighted in this review are the attributes and limitations of these measures in mice in the context of age sensitivity and processes of brain aging. Attributes of these tests include reliability and validity as assessments of learning and memory, well-defined neural substrates, and sensitivity to neural and pharmacological manipulations and disruptions. These tests engage the hippocampus and/or the cerebellum, two structures centrally involved in learning and memory that undergo functional and anatomical changes in normal aging. A test that is less well represented in studies of normal aging, the context pre-exposure facilitation effect (CPFE) in fear conditioning, is described as a method to increase sensitivity of contextual fear conditioning to changes in the hippocampus. Recommendations for increasing the age sensitivity of all measures of normal aging in mice are included, as well as a discussion of the potential of the under-studied CPFE to advance understanding of subtle hippocampus-mediated phenomena.

Keywords: Barnes maze, cerebellum, context pre-exposure facilitation effect, eyeblink classical conditioning, fear conditioning, hippocampus, Morris water maze, rotorod

INTRODUCTION

The concept of age sensitivity in human neuropsychological and psychometric assessment has existed since at least the 1940s (Cattell, 1941, 1943). Early cross-sectional studies (Conrad et al., 1933; Jones and Conrad, 1933) suggested that intellectual abilities peaked in late adolescence and declined thereafter. Later studies that incorporated a longitudinal design challenged this notion, suggesting that some mental abilities were preserved in middle-aged and older adults (Schaie, 1974). A pivotal development in the concept of age-sensitivity was the theory of fluid and crystallized intelligence (Cattell, 1943; Horn and Cattell, 1966, 1967; Horn, 1968, 1975). Using factor analytic techniques, Raymond Cattell and John Horn identified two major components that comprise intelligence. It was the notion of multiple components of intelligence combined with evidence that these factors have different trajectories over the life span that led to the conceptualization of tests that were age sensitive.

A number of behavioral tests of normal aging in animals, including humans, rely to a varying extent on two primary brain regions – the hippocampus and cerebellum. These two structures age at different rates (Woodruff-Pak and Finkbiner, 1995; Green and Woodruff-Pak, 2000; Woodruff-Pak et al., 2009) as a consequence of different mechanisms of normal aging (Woodruff-Pak, 2006; Woodruff-Pak et al., 2010). That the hippocampus and cerebellum age at different rates implies a number of hypotheses about performance differences between young and old animals, which are somewhat parallel to the conclusions of Cattell and Horn about human intelligence. For example, performance on certain tasks should show age-related deficits earlier than others, depending on the neural substrate engaged. Other brain regions are affected by normal aging, though a discussion of these structures is beyond the scope of this review. The behavioral tests included are among the most frequently used in studies of normal aging, thus the focus will be on two of the substrates that support performance in tasks most likely to be implemented in studies of aging.

Behavioral tests such as the Barnes maze, eyeblink classical conditioning (EBCC), fear conditioning, Morris water maze (MWM), and rotorod are consistently used in studies of normal aging. These tests have readily identifiable and well-studied neural substrates and reliably show behavioral evidence of learning. In addition, aged animals are able to complete these tasks, though rates of learning and retention may differ from young animals. The rotorod, Barnes maze, and Morris water maze are primarily conducted with rodents, though human analogs of the MWM utilizing virtual reality exist (Moffat and Resnick, 2002; Berteau-Pavy et al., 2007). Classical fear conditioning can be accomplished in humans as well as rodents, and EBCC has been tested in a number of mammalian species, including humans, non-human primates, cats, rabbits, and rodents. As each test relies on the cerebellum and/or the hippocampus, it is useful to consider different trajectories of aging when evaluating the value of a particular test in terms of refining the body of data on normal aging.

To the best of our knowledge, a comprehensive analysis of the age sensitivity, or the ability to detect age-related deficits in performance, has not been compiled for behavioral tests in mice, the most commonly used mammalian species for studies of aging. The capability to describe the age sensitivity of the tests is valuable when planning experiments on processes of aging. We review the application of these tests to studies of aging and describe the age sensitivity of each test.

UTILITY OF THE MOUSE SPECIES TO INVESTIGATE NORMAL AGING

There are a number of benefits provided by using rodents as a mammalian model of aging. Mice have a relatively short life span (C57BL/6 mice: about 26–28 months; Jucker and Ingram, 1997), and the mouse genome has been sequenced. The potential for genetic manipulation in the mouse has led to an increased interest over the previously favored rat model for investigations of the neurobiology of learning, memory, and aging (Ingram and Jucker, 1999; Vogel et al., 2002). In addition, with the extensive knowledge of the mouse genome, the potential for genetically altered mouse models of aging (both normal and pathological) is unrivaled by other mammalian species. The mouse can be used to study the mechanisms of aging from a number of converging methodologies: behavior, genetics, anatomy, physiology, and histology. Given these advantages, mouse models can be useful for studying normal human aging; however, the primary goal of this review is to compare and contrast the behavioral tests employed to study normal aging in mice. Some behavioral tests included in this review were originally developed for rats (e.g., MWM, Barnes maze) but have been modified to accommodate mice. Mice and rats share sufficient genetic ancestry to be discussed together, although performance on some tasks such as the MWM is not identical, primarily because rats swim better than mice (see Whishaw, 1995; Ingram and Jucker, 1999). The main focus will be the mouse literature, but where mouse data are insufficient or absent, results from other species (rat, rabbit) will be included.

CRITERIA TO EVALUATE AGE SENSITIVITY OF BEHAVIORAL TESTS

Given the wide variety of tests employed to study normal aging in mice, it is useful to develop standard criteria to evaluate age sensitivity. Bartus et al. (1983) created criteria for developing animal models of age-related memory impairment. Their criteria were modified and expanded beyond the original pharmacological scope by Woodruff-Pak (1990). The criteria presented here were determined through an evaluation of the literature but are consistent with both the criteria of Bartus et al. (1983) and Woodruff-Pak (1990). The criteria adopted in this review for evaluating the age sensitivity of behavioral tests of learning and memory include the following:

1. Behavior measured should show reliable deficits in older animals compared to younger animals.
2. The test should avoid confounds from secondary or alternate processes that could account for behavioral differences.
3. The test should avoid food restriction or intense stressors that could weaken older animals and affect performance.
4. Deficits in old animals should mirror those of young animals with experimentally induced lesions to the target neural substrate.
5. Mouse strain variations in the behavior need to be noted, and the behavior should show parallels across multiple species (human and non-human animal).
6. Drugs known to improve behavior in the aged in clinical trials should also improve performance on the test.
7. From a neuroscience perspective, the test should engage neural substrates that support learning and memory (hippocampus and/or cerebellum).

RATIONALE FOR THE FOCUS ON HIPPOCAMPUS AND CEREBELLUM

The hippocampus is essential for declarative forms of learning and memory. As such, it is the brain structure most frequently studied as a learning and memory substrate. Surprisingly, it is challenging to identify behavioral tasks that are solely dependent on the hippocampus. Most often, other brain structures are also normally engaged or essential. The cerebellum is another structure activated or essential in many learning and memory tasks. Even when controls are introduced to remove the motor aspects of the task, the cerebellum is still essential in learning. Thus, the hippocampus and cerebellum are the central brain substrates in this review.

AGING IN THE HIPPOCAMPUS

Loss of hippocampal neurons was at one time thought to be a hallmark of normal aging and associated decreases in learning and memory. With the advent of unbiased stereological techniques, evidence for loss of neurons in the hippocampus was invalidated. Results of studies in humans (West, 1993; West et al., 1994) and rodents (Rasmussen et al., 1996) documented stability in the number of hippocampal pyramidal neurons across the life span. Stereological analysis of pyramidal neurons in the hippocampus of CBA mice aged 4–24 months in our lab replicated previous results demonstrating stability of neuron number over most of the adult mouse life span (Woodruff-Pak et al., 2010).

Hippocampal function does decline in normal aging. Age-related impairment in electrophysiological processes (Barnes, 1999), alterations in synapses (Geinisman et al., 1992), and a decline in neurogenesis (Bondolfi et al., 2004) have been observed. Synaptic inefficiency appears to be a primary cause of age-related performance deficits on tests of learning and memory. Synaptic disruption in the aged hippocampus has been identified in many studies, though this change may be manifested in localized disruptions of hippocampal circuitry (at the subfield level) and not in a global loss of synaptic markers (Nicolle et al., 1999a; Smith et al., 1999). Other potential disruptions of the aged hippocampus may be due to Ca^{2+} dysregulation of adenlyl cyclase (Mons et al., 2004) or changes in neurogenesis (Bondolfi et al., 2004; van Praag et al., 2005). Age-associated decreases in neurogenesis have been reported in the granule cell layer and hilus of the dentate gyrus between 2 and 18 months in C57BL/6 mice (Bondolfi et al., 2004). Changes included a decrease in proliferation but not survival of new neurons. No decline was reported between 18 and 24 months, though other studies have reported progressive decreases in hippocampal

neurogenesis through 24 months (Heine et al., 2004). It is important to note that in the majority of studies, the loss of synapses or decrease in hippocampal efficiency is not present in all aged animals. There is a subpopulation of aged animals that shows impairment on spatial memory tasks, and it is these animals that show reductions in hippocampal plasticity and synapse numbers.

AGING IN THE CEREBELLUM

The cerebellum shows a number of changes in plasticity, cell number, and morphology with age. In an analysis of cerebellar RNA, Popesco et al. (2008) reported a relatively small group of genes affected by normal aging. The age-affected genes appear to be related to lipid biosynthesis, which suggested that changes in conductivity of the cerebellum due to aging are not the result of large-scale cell loss but rather a focused and regulated molecular process. Other research highlights the age-related loss of Purkinje neurons in the cerebellum in humans (Anderson et al., 2003), rats (Larsen et al., 2000), and multiple strains of mouse (Woodruff-Pak, 2006; Woodruff-Pak et al., 2010).

The loss of Purkinje neurons is significantly associated with deficits in learning and memory assessed by 500-ms delay EBCC (Woodruff-Pak, 2006). In addition to a loss of neurons, the morphology of Purkinje cells is altered with age. Older rats have Purkinje cells with a smaller soma volume (Larsen et al., 2000) and defoliation and smaller spiny branchlets (Greenough et al., 1986). A decrease in long-term depression (LTD), indicating reduced synaptic plasticity in the cerebellum has been reported in older (8 and 12 month compared to 4 month) CBA mice (Woodruff-Pak et al., 2010). Finally, evidence suggests that the cerebellum shows age-related deficits earlier than the hippocampus (Woodruff-Pak and Finkbiner, 1995; Woodruff-Pak et al., 2010), which becomes critical when evaluating behavioral tests that rely on both structures.

BEHAVIORAL TESTS AND AGE SENSITIVITY

Each test included in this review (MWM, Barnes maze, rotorod, EBCC, and fear conditioning) is used in the field of aging research and to some extent is supported by the cerebellum and/or the hippocampus. Tests of attention or sensory function are not considered, as the focus of this review is changes in learning and memory across the life span of the mouse. Appetitive tasks, such as the radial arm maze and instrumental learning tests are not included in this review. Tests that rely on food restriction can differentially affect older animals, leaving them too weak to perform. Tests were included that avoid potential confounds such as food restriction that can impair performance of older animals and bias conclusions of age-related deficits in learning and memory.

MORRIS WATER MAZE

The Morris water maze (MWM; **Figure 1**) is a test of spatial ability. It requires the animal to navigate to the location of an escape platform that is hidden under water.

Neural substrates

This test was originally developed to examine the contribution of the hippocampus to navigation and place learning in the rat (Morris et al., 1982). Rats with total aspiration lesions of the hippocampus were impaired on the MWM, showing longer latencies to find the hidden platform and less time swimming in the former location of the platform in a probe retention test (platform removed). Rats with lesions to the hippocampus showed no impairment when the platform was made visible, suggesting that cued learning is not affected. In addition, cognition-enhancing drugs that modulate hippocampal function improve MWM performance in both young rats given pharmacological challenge and old rats that are impaired on the task (Fontana et al., 1995; Yamazaki et al., 1995).

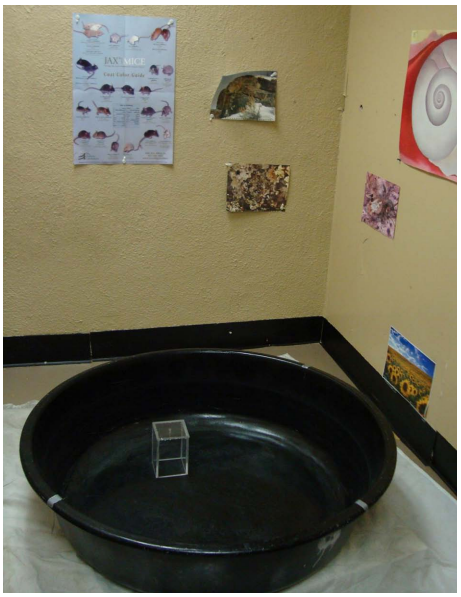

FIGURE 1 |The MWM is a test of spatial learning and memory. Through successive placements into the pool of water, the animal learns to swim to the location of the platform with the aid of distal, extra-maze cues placed around the apparatus. A cued condition where the hidden platform is made visible serves as a control for sensory or motor impairment.

Subsequent research has confirmed the essential role of the hippocampus through lesion studies in mice (Gerlai and Roder, 1996; Logue et al., 1997a) and rats (Gallagher and Nicolle, 1993; van Praag et al., 1998; Gould et al., 2002) and associations between impaired performance and both the functional properties and connectivity of granule cells (Nyffeler et al., 2010). More specifically, the dorsal hippocampus seems to be essential, as lesions to the ventral hippocampus do not impair MWM learning (Bannerman et al., 1999). Though the hippocampus is essential, lesions to the surrounding corticohippocampal circuitry, including the perirhinal, postrhinal, and entorhinal cortices do not impair learning (Burwell et al., 2004). In addition, hippocampus-lesioned rats may still solve the MWM but may use alternate, non-spatial strategies (Pouzet et al., 2002) that involve other brain regions such as the striatum (Miranda et al., 2006). Interventions that increase neurogenesis in the hippocampus, such as voluntary exercise, can also improve spatial-learning ability in the MWM (Kempermann et al., 1997; van Praag et al., 2005).

Another essential brain region for spatial learning in the MWM is the cerebellar cortex. Unilateral removal of a cerebellar hemisphere alters (but does not impair) place learning in DA/HAN rats (Colombel et al., 2004). Mice with altered currents in

cerebellar cortical Purkinje neurons due to knockout of a sodium channel-coding gene showed spatial-learning deficits on the MWM (Woodruff-Pak et al., 2006). These mice were not impaired on the cued, visible platform task, which suggests that differences in acquisition and retention of spatial memory were independent of motor deficits. Other studies have implicated the cerebellum in MWM performance through pharmacological lesions of cerebellar cortex (Dahhaoui et al., 1992), irradiation (Le Marec et al., 1997), and lesions to the pontine and inferior olive cerebellar afferents (Gasbarri et al., 2003). Mutant mouse strains with cerebellar cortical dysfunction such as *pcd*, *Lurcher*, *hot-foot,* and *staggerer* are impaired in acquisition (Goodlett et al., 1992; Lalonde and Strazielle, 2003a). Studies of *Lurcher* mutant mice suggested that the cerebellum is not necessary for learning a spatial task but plays a crucial role in retention of spatial memory (Hilber et al., 1998).

Age-related deficits

The MWM is one of the most widely used behavioral tests in studies of normal aging. The task was initially used to study aging in rats. Age deficits in the acquisition of the hidden platform location have been reported in a number of strains including Fisher 344 (Frick et al., 1995), Sprague-Dawley (Gage et al., 1984), Long–Evans (Gallagher and Burwell, 1989), dark agouti (Nyffeler et al., 2010), and Wistar (Miyagawa et al., 1998). Within these strains there have been varying reports of the earliest detectable age deficits in the MWM, though generally by 24–27 months rats show significant impairment. Some studies have reported impairment in acquisition at 18–22 months in Fisher 344 rats (Shukitt-Hale et al., 1998) or as early as 11 months in the same strain (Frick et al., 1995). Strain affects the age of onset of performance deficits. For example, 28-month Long–Evans rats showed no impairment in spatial memory while 28-month Fisher 344 rats were severely impaired (Lindner and Schallert, 1988).

The MWM has been adapted for mice, though a number of issues arise when attempting to compare mouse and rat performance on the test. Mice require more trials for acquisition than rats (Magnusson et al., 2003) and generally perform less well than rats (Whishaw, 1995; Whishaw and Tomie, 1996; Prut et al., 2007). Mice are more sensitive to cold water used in the test (Bellush et al., 1996) and may not be as well adapted to swimming as rats (Prut et al., 2007). In a direct comparison of C57BL/6 mice and Long–Evans rats, Whishaw and Tomie (1996) reported impairments in mice relative to rats in terms of place learning when the MWM was used, though performance was equal when place learning was carried out on a dry, radial arm maze. The investigators interpreted these results to indicate that rats are better adapted to swimming in water than mice. Struggling with the motor aspects of swimming may detract from the ability to orient to cues, thus reducing the capacity to perform in the water maze.

Despite the obvious differences between mouse and rat performance in the MWM, a number of studies have included mice to evaluate age differences in spatial memory. Age deficits in mice have been reported in C57BL/6 (Bellush et al., 1996; Magnusson et al., 2003; Harburger et al., 2007), CD1 (Gerlai and Roder, 1996; Francia et al., 2006), NMRI (Gower and Lamberty, 1993), and numerous other inbred and hybrid strains (Markowska et al., 1998; Prut et al., 2007; Harrison et al., 2009a). Age differences have been reported across a number of strains with varying ages of earliest detection. In C57BL/6 mice, age deficits are seen at 24 (Bellush et al., 1996; Magnusson et al., 2003), 18 (van Praag et al., 2005), 15 (Harburger et al., 2007), and as early as 12 months (Wong and Brown, 2007). Gower and Lamberty (1993) reported that from middle age (11–12 months) on (to 22 months), deficits in acquisition and retention of spatial memory are apparent and independent of spontaneous locomotor activity, sensorimotor, or emotionality deficits.

There is greater variability across mouse strains on the MWM compared to rats. This perhaps is a reflection of the much larger variety of inbred, outbred, hybrid, and genetically engineered mouse strains used in aging research. Owen et al. (1997) conducted an extensive comparison of MWM performance in 12 mouse strains commonly used for genetic backgrounds and seven hybrid strains. Only the C57BL/6J, C57BL/10J, and 129/SvevTac strains were capable of complex learning across multiple tasks, which included the MWM and fear conditioning. Generally, the albino strains (FVB/NJ, SJL/J, A/J, and Bub/Bnj) tested did not demonstrate evidence of learning on the MWM task, though deficits in visual acuity could explain acquisition deficits for some (e.g., FVB/NJ). Other strains such as the 129/SvJ and DBA/2J-I showed decreasing latencies to escape the pool but no clear evidence of spatial learning when tested in the probe task. Additional research has shown that strain-dependent differences in visual acuity can confound MWM performance. In a comparison of C57BL/6 and DBA (which develop glaucoma-like symptoms) mice, Wong and Brown (2007) showed no strain difference in MWM performance or visual acuity at 6 months. By 12 months, and continuing through 24 months, DBA mice showed significant impairment on both the MWM and visual acuity tasks. This study highlights contributions of mouse strain to MWM performance and demonstrates that different strains show different developmental trajectories of spatial-learning ability.

Age sensitivity

The MWM affords a number of benefits in terms of age sensitivity. It does not involve food restriction or the administration of shock to encourage participation of animals (Gage et al., 1984). Memory deficits can be detected independent of sensorimotor impairments (Gage et al., 1989; Gallagher and Burwell, 1989; Miyagawa et al., 1998) through the visible platform, a cued version of the task that is sensitive to deficits in vision and motor function. Several studies have reported age differences in both rats and mice in the acquisition and retention of spatial memory with no group differences seen in the visible platform task (Gerlai and Roder, 1996; Harrison et al., 2009a).

The effects of aging on both performance of the MWM and the neural substrates involved have been corroborated in studies of young lesioned animals. Aged animals (presumably which have endured changes to the hippocampus over time) and young hippocampus-lesioned animals show a similar pattern of deficits in spatial memory (Gallagher and Nicolle, 1993).

Alterations in methodology to improve the age sensitivity of the MWM have been suggested. Many of these changes involve increasing the difficulty of the task, such as incorporating a variable interval probe where the platform is unavailable for a variable amount of time but then is raised (Frick et al., 1995), combined measures of a learning index (de Fiebre et al., 2006) or emphasis

on dependent measures such as platform crossings in the probe test (Markowska et al., 1998). Crossing the platform location is more difficult than swimming or covering distance in the trained quadrant, the other common measures of the probe test, because it requires a precise spatial memory of the hidden platform location. The increase in difficulty affords a greater ability to detect age deficits. At the very least, multiple measures should be used including latency to escape, distance traveled, and probe components to evaluate accurately age-related deficits (de Fiebre et al., 2006). Using multiple measures becomes especially important in cases where differences in swimming speed arise, as these differences can bias the latency measure (Klapdor and van der Staay, 1996). The combination of a number of measures standard to the MWM with some of the more difficult measures recently applied is consistent with the notion that multiple parameters provide for greater sensitivity in detecting the progression of spatial memory deficits as a function of age (Gallagher et al., 1993; Klapdor and van der Staay, 1996).

Several limitations of the MWM have been noted which may reduce the ability to detect differences in learning and memory independent of confounding factors. Mice take longer to learn the task and may struggle with swimming, thus reducing the ability to compile a spatial map of the pool (Whishaw and Tomie, 1996). Impairment as a function of poor swimming ability may be mistaken for impairment in spatial memory capacity. Differences in swim speed and visual acuity can bias the latency measure of spatial memory acquisition (Klapdor and van der Staay, 1996; Wong and Brown, 2007), though visual acuity is generally controlled for by the visible platform task. Strain and age differences in swim speed have been reported (Klapdor and van der Staay, 1996), though generally age differences in swim speed are not (Francia et al., 2006; Harburger et al., 2007; Harrison et al., 2009a). As long as multiple dependent variables are considered for acquisition (latency, distance traveled) and retention (platform crossings, swim time) of spatial memory, confounds can generally be avoided. Overreliance on one measure at the expense of others can undermine conclusions regarding age differences in spatial memory.

One of the greatest limitations of the MWM that may affect age sensitivity is the stressful nature (for both mice and rats) of the paradigm. Stress suppresses neurogenesis in the hippocampus, and repeated stress can cause atrophy of hippocampal CA3 pyramidal neurons resulting in a decrease in spine density and dendritic arborizations (McEwen and Magarinos, 2001; Rodrigues et al., 2009). The hippocampus is particularly sensitive to increases in stress hormones, since it expresses receptors for circulating adrenal steroids (McEwen et al., 1968). Aguilar-Valles et al. (2005) reported a strong stress response on day 1 in Wistar rats, though gradually the stress response attenuated. Most importantly, corticosterone concentration was negatively correlated with learning ability during the first 3 days of training. Other research confirms the stressful nature of the MWM and the negative impact of glucocorticoids on performance (Barrett et al., 2009; Harrison et al., 2009b). Behavioral tests that assess spatial memory in the absence of swimming-induced stress may be more sensitive to age differences in learning capacity.

In an aged population of rodents, not all of the oldest animals are impaired when tested on the MWM (Gage et al., 1984, 1989; Gallagher and Burwell, 1989; Gower and Lamberty, 1993; Lee et al., 1994; Miyagawa et al., 1998; Nicolle et al., 1999b). A subgroup of "good" old performers shows spatial memory abilities equal to that of young animals (Gallagher and Burwell, 1989). Old animals that perform as well as young animals also have comparable levels of neurogenesis in the dentate gyrus and elevated levels compared to their chronologically same-aged but poor performing littermates. Heterogeneity in aging research incorporating the MWM is often reported in the rat (Miyagawa et al., 1998; Nicolle et al., 1999b) and to a lesser extent in the mouse (Gower and Lamberty, 1993). It may become a limitation when "good" and "poor" performing old groups are not identified, as there is likely some underlying difference between the two groups that may be differentially affected by experimental manipulations.

The MWM is not a "pure" hippocampus-dependent task. Differential rates of aging in other structures such as cerebellar cortex preclude interpretations about hippocampal aging alone as underlying spatial memory deficits. The cerebellar cortex may be affected by aging before the hippocampus is affected (Woodruff-Pak et al., 2010); therefore, aging in the cerebellum may initiate age-related deficits in spatial memory performance.

The MWM satisfies four of the criteria to evaluate age sensitivity. Age-related deficits are reported in both mice and rats (Criterion 1); though there are some conflicting reports that suggest deficits associated with normal aging may not be detected by the task (see **Table 1**). These results depend on the type of memory assessed and the species used. Hippocampal and cerebellar damage in young rats and mice mirrors the behavioral deficits of older animals (Criterion 4), whereas drugs that have been shown to improve hippocampal function in older animals enhance spatial memory ability (Criterion 6). There are significant strain differences in MWM performance, and the task cannot be termed purely hippocampal, as cerebellar cortex is also essential (Criteria 5 and 7). A human analog of the MWM does exist, but sufficient data regarding human performance during aging have not been collected and the neural substrates have not been mapped for this task in humans. Finally, a major confound in the MWM is the stressful nature of the swim task, which could bias interpretation of age-related differences (Criteria 2 and 3)

BARNES MAZE

The circular platform, or Barnes maze (**Figure 2**), is similar to the MWM in that it is a spatial navigation task. The rodent learns to locate an escape tunnel.

Neural substrates

The Barnes maze is dependent on the integrity of the hippocampus. Performance on the Barnes maze is impaired when extramaze spatial cues are removed in that animals do not improve across a number of days of testing (Barnes et al., 1980; Pompl et al., 1999). Learning occurs when visual cues are presented to the animal. Mice show a strong preference for using spatial cues to solve the maze (Harrison et al., 2006). In addition, disruptions to the hippocampal formation are associated with deficits in Barnes maze performance, including early postnatal maternal separation (Fabricius et al., 2008) chronic stress (McLay et al., 1998), transgenic Alzheimer's disease mouse models (Pompl et al., 1999), inactivation by drug infusion (Poucet et al., 1991), lesions of the dentate gyrus (McNaughton et al., 1986), or the loss of septo-hippocampal basal

Table 1 | Morris water maze: age sensitivity.

Study	Species/strain	Age (months)	Age deficits (months)	Mirror lesions in young	Confounds/strain differences	Effect of cognition-enhancing drugs	Cerebellum-sensitive	Hippocampus-sensitive
Bellush et al. (1996)	C57BL/6	4, 19, 24	19+ (retention)	Yes	Deficits could be due to sensitivity to cold water			Yes
Colombel et al. (2004)	DA/HAN rat	3		Cerebellar hemispher-ectomy impairs acquisition			Yes	
de Fiebre et al. (2006)	C57BL/6	6, 16, 24	24	Yes				Yes
Fontana et al. (1995)	Wistar rat	4, 23	23	Yes		Serotonin receptor agonist rescued deficits		Yes
Francia et al. (2006)	CD1	5, 13	13	Yes				Yes
Gerlai and Roder (1996)	CD1	3, 16	16	Yes				Yes
Gower and Lamberty (1993)	NMRI	3, 6, 9, 11–12, 17, 22	11–12+	Yes	Not due to locomotor, sensorimotor or emotionality deficits			Yes
Harburger et al. (2007)	C57BL/6	3, 15, 21	15+	Yes				Yes
Harrison et al. (2009a)	C57BL/6	12, 24	24	Yes				Yes
Logue et al. (1997a,b)	C57BL/6, DBA/2, B6D2F1 hybrid	2–5		Hippocampal lesions impair acquisition	DBA non-lesioned did not learn task			Yes
Magnusson et al. (2003)	C57BL/6	3, 10, 24	10+ (immed. WM); 26 (delay WM); 26 (Ref. Mem)	Yes				Yes
Owen et al. (1997)	12 inbred and seven hybrid strains	2–3			Good: C57, hybrids; poor: 129/Sv, DBA, BALB, LP			
van Praag et al. (2005)	C57BL/6	3, 18	18	Yes				Yes
Vogel et al. (2002)	C57BL/6	4, 12, 18	None	No			No	
Wong and Brown (2007)	C57BL/6, DBA/2	6, 12, 18, 24	C57: 12+; DBA: None	Yes	6: No strain diffs; 12+ mo: C57 > DBA; DBA show visual deficits			Yes
Woodruff-Pak et al. (2006)	*Scn8a* KO and C57BL/6	5		Abnormal firing in cerebellar Purkinje cells impairs acquisition			Yes	
Yamazaki et al. (1995)	Fisher 344 rat	3, 24–26	24–26	Yes		Physostig-mine had no effect; Cholinergic activity modulator rescued deficits		Yes

Species is mouse unless otherwise specified. C57 = C57BL/6.

FIGURE 2 | The Barnes maze is a test of spatial memory. The apparatus is a large circular platform with holes spaced evenly around the periphery. The animal is placed in the middle of the platform and is motivated to escape the flat, open surface (necessity of escape is accentuated by bright flood lights shined on the platform). Using spatial cues placed around the platform, the animal learns the location of the single hole, which contains a darkened escape tunnel.

forebrain cholinergic neuron projections (Moreau et al., 2008). In a rodent model of cerebral ischemia, impaired performance was significantly correlated with cell loss in the hippocampus (Milani et al., 1998).Given the role of the cerebellar cortex in supporting MWM performance, it is possible that this structure is involved in the Barnes maze, though the contribution of the cerebellum has not been described. To date, no study has examined Barnes maze performance in cerebellar cortically lesioned or transgenic animals. Further research is necessary to test the hypothesis that the cerebellar cortex is involved in Barnes maze performance.

Age-related deficits

The Barnes maze was originally constructed for rats, and much of the research using the test utilizes this species. Older rats show impairment on dependent measures of the Barnes maze including latency to reach the goal/escape, the number of errors made (choosing the incorrect hole), speed and distance covered (Barnes, 1979; Barnes et al., 1980; McLay et al., 1999). Barnes (1979) originally reported that senescent Long–Evans rats (28–34 months) were impaired relative to younger rats (10–16 months) on all of the dependent measures. In addition, the older rats were impaired on reversal trials, in which they were required to learn the new location of the escape hole.

Very few studies have employed the Barnes maze to examine age-related deficits in mice. Deficits have been reported in C57BL/6 mice as early as 12 months (Bach et al., 1999). Older animals (12 and 18 month) made more errors than younger (3 and 6 month) and relied more on a serial search strategy rather than a spatial strategy. This is contrary to the original reports of Barnes (1979) in which older rats were able to switch to a spatial strategy. Other studies utilizing the Barnes maze and older mice have not compared multiple ages (Prut et al., 2007). To date, it does not appear that there has been a systematic evaluation of Barnes maze performance across the entire life span of the mouse.

Though few studies of aging mice feature the Barnes maze, a large number of studies have suggested that strain differences in spatial abilities exist (Nguyen et al., 2000; Koopmans et al., 2003). Young adult C57BL/6 mice outperformed CD1 mice on the Barnes maze; however, CD1 mice were superior on the MWM (Patil et al., 2009). Despite the fact that both the MWM and Barnes maze measure spatial memory, results suggest that the two tests do not measure completely overlapping abilities. Koopmans et al. (2003) also reported the behavioral superiority of C57BL/6 mice on the Barnes maze when compared to 129/Sv, BALB/c and Swiss mice. Together, these studies suggest two alternative conclusions: (1) that C57BL/6 mice (and genetic mutants built on this background) are ideal for aging studies investigating spatial memory with the Barnes maze due to their ability to learn the task relative to other strains, or (2) that the superiority of performance of this particular strain of mouse may mask any deficits seen in normal aging.

Age sensitivity

A limitation of the Barnes maze is that mice do not perform in the same manner as rats (Koopmans et al., 2003). Mice may not always train correctly on the task. They hesitate around the goal hole and explore other holes instead of escaping. These behaviors inflate the measured variables of the test, including latency to escape, distance covered, and number of errors made. Positive reinforcement (i.e., sugar in the escape chamber) appears to improve performance of mice on this task (Grootendorst et al., 2001), though this strategy undermines the original elegance of the Barnes maze that required no stress or food reinforcers to elicit the behavior. To avoid the need for reinforcers, Koopmans et al. (2003) used a modified version of the Barnes maze. Instead of requiring mice to climb through a hole and into an escape tunnel under the board, the investigators placed holes in the wall surrounding the platform area. Mice were much more likely to walk through the holes in the wall surrounding the platform than to climb down into an escape hole.

Instead of altering the construction of the maze, researchers have suggested other methods to control the bias created in the standard variables by the exploratory behavior of mice. Harrison et al. (2006) calculated latency, path length, and number of errors, but only until the first encounter of the escape hole. Other researchers have adopted this solution (Patil et al., 2009) as it is a better indication of whether the mouse learned the location of the escape hole and can show performance differences that are not captured by total path length, total latency, and total errors.

The most important characteristic that distinguishes the Barnes maze from the MWM is stress (Sternberg et al., 1992; Barrett et al., 2009; Harrison et al., 2009b). Both the Barnes maze and MWM

increase levels of stress hormones compared to non-tested controls, but plasma corticosterone levels were significantly higher in the MWM compared to the Barnes maze (Harrison et al., 2009b). On the MWM (but not the Barnes maze), behavioral performance was inversely correlated with corticosterone levels. The original rationale of Barnes (1979) in creating the maze was that it involved no stressors, shocks, or food restriction, all of which could weaken or impair older rats and confound measures of age differences in learning and memory. Therefore, the Barnes maze seems to be sensitive to similar disruptions in hippocampal function as the MWM but is less physically demanding and less prone to confounding variables such as stress. These qualities would suggest that the Barnes maze should be more sensitive to aging in the hippocampus than the MWM.

Aged rats are impaired in aspects of spatial memory assessed by the Barnes maze and similar results have been reported in mouse populations (Criterion 1), though the mouse literature is less extensive (see **Table 2**). The Barnes maze may elevate stress, but increased stress is not associated with performance deficits (Criteria 2 and 3). This is not true for the MWM, as stress is both greater and associated with impaired performance. Lesions to the hippocampus of young rats lead to performance deficits similar to those of aged rats (Criterion 4). The Barnes maze, much like the MWM, seems to be affected by strain, as performance varies across a number of mouse and rat strains (Criterion 5). The effect of cognition-enhancing drugs on Barnes maze performance has not yet been sufficiently explored as preliminary studies (Ambrée et al., 2009) have generally focused on pathological rather than normal aging (Criterion 6). However, dopamine D_1/D_5 receptor agonists rescue both performance and electrophysiological deficits in aged (12–18 month) C57BL/6 mice compared to young (3–6 month) mice (Bach et al., 1999). Finally, the contribution of the cerebellum to spatial memory as measured by the Barnes maze has not

Table 2 | Barnes maze: age sensitivity.

Study	Species/strain	Age (months)	Age deficits (months)	Mirror lesions in young	Confounds/strain differences	Effect of cognition-enhancing drugs	Cerebellum-sensitive	Hippocampus-sensitive
Arendash and King (2002)	Tg2576, C57BL/6	3, 14, 19	C57: 14 < 3, 19	Yes	May involve sensorimotor and/or anxiety components			No
Bach et al. (1999)	C57BL/6	3, 6, 12, 18	12, 18	Yes		Dopamine D_1/D_5 agonists rescued deficits		Yes
Barnes (1979)	Long–Evans rat	10–16, 28–34	28–34	Yes				Yes
Barnes et al. (1980)	Long–Evans rat	9, 26	26	Yes				Yes
Barnes et al. (1991)	Fisher 344 rat	6, 27	27	Yes				Yes
Barrett et al. (2009)	Sprague-Dawley, dark agouti rat	6, 11, 14, 17, 20, 26	Agouti: 11+; Sprague-Dawley: 14+	Yes	Sprague-Dawley > dark agouti			Yes
Harrison et al. (2009a,b)	C57BL/6	2			Inc corticosteronenot correlated with performance			
King and Arendash (2002)	Tg2576, C57BL/6	3, 14, 19	C57: 14 < 3, 19	Yes	Aged B6 may not show deficits in spatial memory			No
Koopmans et al. (2003)	129/Sv, C57BL/6, BALB/c, Swiss	3			Hesitate at goal C57 > 129 > BALB > Swiss			
McLay et al. (1999)	Fisher 344 × Brown Norway rat	3, 6, 25	25	Yes				Yes
Patil et al. (2009)	C57BL/6, CD1	3			C57 > CD1			

Species is mouse unless otherwise specified. C57 = C57BL/6.

yet been investigated, so it is premature to declare the test as purely hippocampal (Criterion 7). The Barnes maze satisfies four of the criteria for the evaluation of age-sensitivity. Additional research is needed to examine the impact of cognition-enhancing drugs, the influence of mouse strain, and the contribution of the cerebellum to the task.

ROTOROD

Rotorod is a task assessing balance, coordination, and motor learning (**Figure 3**).

Neural substrates

The cerebellum is essential for sustaining rotorod performance (Lalonde et al., 1995; Lalonde and Strazielle, 2003b). Studies conducted in mice with mutations affecting cerebellar Purkinje cells implicate the cerebellum as essential for the task, but the degree to which cerebellar atrophy affects the rotorod is unclear. *Lurcher* mice, a strain with degenerated cerebellar granule and Purkinje cells, are able to learn the rotorod task, but show shorter latencies to fall than control mice (Lalonde et al., 1995). Two other mutant strains, *staggerer* (massive degeneration of granule and Purkinje cells) and *hot-foot* (defective innervation of Purkinje cells but not massive degeneration) showed shorter latencies to fall than the *Lurcher* mice, suggesting differences in rotorod performance from different types of cerebellar atrophy. Other research suggests that the cerebellar cortex is critical for the coordinated whole body movements needed to perform on the rotorod but not for stationary motor tasks such as vertical and tilted grids and a coat-hanger task (Le Marec and Lalonde, 1997). A threshold for the number of Purkinje cells necessary to show evidence of motor deficits has been proposed through the performance of chimeric (individual animals in the litter have different numbers of Purkinje cells) mice (Martin et al., 2003). Performance on the rotorod task is clearly linked to the underlying cerebellar structure.

FIGURE 3 | The rotorod is a motor task that assesses coordination and learning. It involves a rotating beam on which the animal is placed. The animal must move forward in a coordinated manner to avoid falling.

Age-related deficits

The rotorod is widely used to test motor coordination and learning at various ages from as young as 19-days old (Bearzatto et al., 2005), through adulthood (Thouvarecq et al., 2001) and into old age (Ingram et al., 1987). Older mice show impairment on both the standard and accelerating rotorod paradigms, and age deficits are also seen in older rats (Bickford, 1995; Shukitt-Hale et al., 1998). Despite the general finding that age-related deficits are observed on this test, there remains considerable disagreement about the age at which deficits become apparent. A number of studies have reported that deficits appear around 12–13 months in C57BL/6 mice (Thouvarecq et al., 2001; Vogel et al., 2002), though deficits have been reported as early as 7 months (Fetsko et al., 2005; Serradj and Jamon, 2007). Depending on the study, paradigm, and species, deficits become progressively worse with older age, though this is not reported in all cases.

AGE SENSITIVITY

The rotorod is useful in detecting age-related differences in cerebellar function, however, different species of mouse perform at different levels, and strain differences do not remain consistent throughout development (Crawley et al., 1997; Bearzatto et al., 2005). For example, C57BL/6 mice outperformed 129/Sv mice at multiple ages tested and showed a significant decrease in motor learning beginning at 7 months, which declined further at 13 months (Serradj and Jamon, 2007). 129/Sv mice showed no real improvements in motor learning from 1 to 3 months and showed a slight (but non-significant) decrease at the older ages. Both of these strains are utilized in the creation of mutant mouse strains, including models of accelerated aging and age-related disease. Strain differences in rotorod performance have been recognized (Logue et al., 1997b; Contet et al., 2001; Voikar et al., 2001; Serradj and Jamon, 2007), and different strains of mice show different age-related changes in performance (Ingram and Jucker, 1999). When considering the utility of the rotorod in assessing age-related changes in motor learning, strain differences could present a problem of interpretation – motor impairment may vary at the same age in testing due to the relative genetic contributions of the strain(s) involved.

An additional issue that affects the sensitivity of the rotorod is that performance requires a number of components – balance, coordination, motor skills, and learning skills – that make interpretation of results difficult (Serradj and Jamon, 2007). The current paradigm cannot identify the source of deficits; any one (or combination) of the abilities could be responsible. Thouvarecq et al. (2001) have argued that the rotorod measures motor learning and is either insensitive to or does not measure motor skills. Body weight may pose a particular problem to the age sensitivity of the rotorod, as some studies have shown that it is negatively correlated with performance (Ingram, 1983). Weight could confound conclusions about age-related changes in the cerebellum contributing to deficits seen in performance, though this correlation has not always been replicated (Ingram et al., 1987).

The rotorod satisfies some, but not all, of the criteria to evaluate age-sensitivity. The test is able to detect age-related deficits in motor learning in both rats and mice (Criteria 1 and 5), but has not been tested in other species (see **Table 3**). Performance may be confounded by weight and could be accounted for by a number of components of motor function that cannot be differentiated (Criterion 2). Any stress caused by running on the rotating beam is not sufficient to affect older animals and prohibit completion of the task (Criterion 3). A large body of lesion and mutant strain research has confirmed that the rotorod relies on the intact cerebellum and can be considered a purely cerebellar task (Criteria 4 and 7). As for the sixth criterion, drugs that improve learning and memory in clinical trials have not been applied to the rotorod, though animals modeling the massive loss of dopaminergic neurons seen in Parkinson's disease show improved performance when given coenzyme Q_{10} (Somayajulu-Nitu et al., 2009) or selective dopamine D_3 receptor antagonists (Mela et al., 2010).

EYEBLINK CLASSICAL CONDITIONING

Eyeblink classical conditioning is a test of associative learning in which a neutral stimulus is paired with a blink reflex-emitting stimulus until the neutral stimulus elicits the reflex or a partial reflex (**Figure 4**).

Neural substrates

The circuitry required to support the formation of conditioned responses in EBCC has been almost entirely defined and parallels exist in neurobiology and behavior across a number of mammalian species (Woodruff-Pak and Steinmetz, 2000a,b; Christian

Table 3 | Rotorod: age sensitivity.

Study	Species/ strain	Age (months)	Age deficits (months)	Mirror lesions in young	Confounds/ strain differences	Effect of cognition-enhancing drugs	Cerebellum-sensitive	Hippocampus-sensitive
Bearzatto et al. (2005)	C57BL/6, NMRI, 129B6 F2	<1, 3			C57 < NMRI and 129B6 at PND 19 C57 and NMRI > 129B6 at 3 months; different rates of learning in strains		Yes	
Fetsko et al. (2005)	C57BL/6	1, 3, 7, 10, 12, 18, 23	7	Yes			Yes	
Ingram et al. (1987)	C3B10RF	11–15, 31–35	31–35	Yes	Weight confound		Yes	
Le Marec et al. (1997)	*Lurcher* mutant	3–4		Loss of Purkinje cells impairs learning			Yes	
Mela et al. (2010)	Sprague-Dawley rat					Dopamine D_3 agonist rescues deficits in Parkinson's model		
Serradj and Jamon (2007)	129/Sv and C57BL/6	1, 3, 7, 13	C57–7; 129 – None	Yes	C57 > 129 at all ages; 129 poor learners		Yes	
Shukitt-Hale et al. (1998)	Fisher 344 rat	6, 12, 15, 18, 22	12+	Yes			Yes	
Somayajulu-Nitu et al. (2009)	Long–Evans rat	3				Coenzyme Q_{10} rescues deficits in Parkinson's model		
Thouvarecq et al. (2001)	C57BL/6	3, 12, 20	12, 20	Yes			Yes	
Vogel et al. (2002)	C57BL/6	4, 12, 18	12, 18	Yes			Yes	

Species is mouse unless otherwise specified. C57 = C57BL/6.

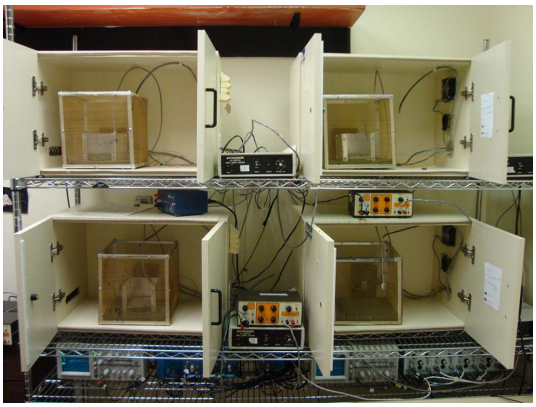

FIGURE 4 | Eyeblink classical conditioning is a form of associative learning in which a previously neutral stimulus such as a tone or light (CS) is presented briefly before a blink-inducing unconditioned stimulus (US), such as a puff of air to the eye or a shock to the muscles around the eye. In the standard delay paradigm, the CS and US overlap, and coterminate. After repeated pairings, the animal learns to associate the stimuli and begins to blink to the CS before the onset of the US – a behavior termed conditioned response (CR).

and Thompson 2003). The essential site of plasticity is the interpositus nucleus of the cerebellum, ipsilateral to the conditioned eye (Thompson, 1986). Information about the US carried in the climbing fibers projecting from the inferior olive and the CS carried by the mossy fibers from the pontine nucleus converge on the Purkinje cells of the cerebellar cortex and the interpositus nucleus leading to the plasticity that supports learning. Purkinje cells, which are large, inhibitory neurons, provide the only efferent input to the interpositus nucleus from the cerebellar cortex and affect the rate of conditioning, though they are not essential for the formation of a CR (Christian and Thompson, 2003; Woodruff-Pak and Disterhoft, 2008). Purkinje cell degeneration (*pcd*) mice that lack any output from the cerebellar cortex to deep nuclei are profoundly impaired in acquisition of delay EBCC (Chen et al., 1996; Woodruff-Pak and Disterhoft, 2008), though they are still able to acquire some CRs. Lesions to the interpositus nucleus, however, completely abolish the CR (Woodruff-Pak et al., 1985; Thompson, 1986; Chen et al., 1999). The hippocampus is not required for delay conditioning, though it can modulate the rate of conditioning (Berger et al., 1976, 1983; Thompson et al., 1983; Christian and Thompson, 2003). Lesions to the hippocampus do not impair acquisition of CRs (Schmaltz and Theios, 1972; Solomon and Moore, 1975), whereas functional disruption causes impairment (Solomon et al., 1983). Finally, a cerebellar cortical LTD-like process is required to support normal delay conditioning (Ito, 1989; Linden and Connor, 1995; Christian and Thompson, 2003; Woodruff-Pak and Disterhoft, 2008).

Trace eyeblink conditioning is distinct from delay conditioning in that the CS and US are separated by a stimulus free interval. The essential site of plasticity is still the interpositus nucleus (Woodruff-Pak et al., 1985), though to process the stimulus free trace period, forebrain areas such as the hippocampus (Kim et al., 1995; Weiss et al., 1999; Tseng et al., 2004) and prefrontal cortex (Kronforst-Collins and Disterhoft, 1998; Weible et al., 2000) are necessary. Scopolamine administration at doses low enough to have little effect on delay conditioning prevent acquisition of the trace CR, whereas lesions to the hippocampus after training abolish the CR (Kaneko and Thompson, 1997), though this retrograde amnesia is time-limited (Kim et al., 1995). Initial work in rabbits suggested that the cerebellar cortex is involved in trace conditioning (Woodruff-Pak et al., 1985), though studies using transgenic mice demonstrated that trace conditioning remained normal despite disruptions of the cerebellar cortex (Kishimoto et al., 2001a,b, 2002). Forebrain structures bypass the cerebellar cortex in trace conditioning by bridging the temporal gap through pontine–cerebellar nuclear connections (Woodruff-Pak and Disterhoft, 2008). Further evidence that the cerebellar cortex may not be necessary for trace conditioning comes from a study of homozygous *pcd* mice (Brown et al., 2010). These mice, with a complete loss of cerebellar cortical Purkinje cells were not impaired on trace conditioning. The cerebellar cortex does not appear to be engaged in trace conditioning, although it supports a normal rate of acquisition in delay eyeblink conditioning.

Age-related deficits

The ability to perform EBCC declines with age. This result has been replicated in a variety of mammalian species such as humans (Solomon et al., 1989; Woodruff-Pak and Thompson, 1988), rabbits (Thompson et al., 1996; Woodruff-Pak et al., 2001), mice (Woodruff-Pak, 2006), and cats (Harrison and Buchwald 1983). In humans, deficits begin to emerge in the decade of the 40s, performance declines significantly in the 50s, and remains relatively stable through the 80s (Woodruff-Pak and Thompson, 1988; Woodruff-Pak and Jaeger, 1998).

In rabbits, the developmental trajectory of age deficits mirrors that of humans, with differences seen first around middle age. Woodruff-Pak et al. (1987) reported increases in the number of trials to reach criterion in older rabbits (30 and 45 months) compared to younger (3 month) in the trace paradigm, though not all older animals were impaired. The estimated life expectancy for a rabbit is about 8 years (Fox, 1980) and based on declines seen in reproductive capacity around 18–24 months, the impaired older rabbits would be comparable to humans aged 40–50 years – precisely the age range identified by Woodruff-Pak and Thompson (1988).

Declines in reproductive capacity (decrease in litter size) in the mouse begin around 8 months (Biggers et al., 1962; Pritchett and Taft, 2006), suggesting that at this age mice approximate rabbits 18-months-old and middle-aged humans. In the 250-ms delay paradigm, mice aged 9, 12, and 18 months were impaired relative to mice aged 4 months, taking longer to develop CRs (Vogel et al., 2002). These results were not entirely supported in another study (Kishimoto et al., 2001c) that compared the same strain of mouse (C57BL/6) in a very similar paradigm (252-ms delay). These authors reported impaired EBCC at the oldest age tested (21–22 months) but not at middle age (11–12 months). Other studies have shown no significant differences in performance in C57BL/6 mice at 12 months (Woodruff-Pak, 2006) in the 500-ms delay paradigm, though conclusions regarding performance of C57BL/6 mice may be confounded by age-related loss of hearing around middle age (Ouagazzal et al., 2006). Auditory acuity remains relatively intact over the life span in CBA mice (Ohlemiller, 2009). We recently reported that CBA mice aged 24 months were significantly impaired in the 500-ms delay paradigm relative to 4-month-old mice (Woodruff-Pak et al.,

2010). In addition, unbiased stereological estimation of Purkinje neurons showed age-related decreases at both 18 and 24 months. Other research has confirmed the age-related loss of Purkinje cells in mice (Woodruff-Pak, 2006) and rabbits (Woodruff-Pak et al., 1990) and the relation to age deficits in EBCC.

Age sensitivity

The age sensitivity of EBCC is apparent in its wide-ranging applicability, where age-related deficits have been reported in a number of species including mice (Kishimoto et al., 2001c; Vogel et al., 2002; Woodruff-Pak, 2006; Woodruff-Pak et al., 2010), rats (Weiss and Thompson, 1992), rabbits (Woodruff-Pak et al., 1987, 2007a; Woodruff-Pak, 1988), and humans (Woodruff-Pak and Thompson, 1988; Solomon et al., 1989; Woodruff-Pak and Jaeger, 1998). Due to the extremely well-studied neural substrates, a number of cross-species commonalities have been reported in terms of neurobiology and behavioral performance. In addition, EBCC performance deficits with age have been associated with Purkinje cells, one of the few neurons in the CNS that show significant loss with normal aging (Larsen et al., 2000; Anderson et al., 2003; Woodruff-Pak, 2006; Woodruff-Pak et al., 2010), thus providing an identifiable neural substrate for age deficits. The advantage of EBCC over many of the tests considered here is that it can be accomplished in human subjects, therefore providing a translational utility across human and non-human species. Classical fear conditioning can be conducted in human populations (Alvarez et al., 2008), although some investigators are reluctant to employ a paradigm that requires the application of shock to humans. A parallel assessment for spatial learning and memory in rodents (Morris water maze and Barnes maze) that uses virtual reality has been elaborated recently and tested in children and older adults (Haley and Raber, 2011). With EBCC, the power of conclusions regarding age-related performance deficits in humans is only strengthened by the similarity in performance across species. Finally, this paradigm provides the ability to take advantage of a number of species-specific experimental designs, including the short life span and potential for genetically altered mice, as well as the larger brain size and better adaptability to restraint of rabbits (Woodruff-Pak, 1988).

Woodruff-Pak (1990) reviewed the classically conditioned nictitating-membrane response in rabbits as a model system for studying learning, memory and aging using criteria similar to those suggested in this review to examine the sensitivity of behavioral tests in the aging mouse. It was argued that data to support a criterion similar to Criterion 6 here, that pharmacological agents would ameliorate age-related deficits in performance, were promising but still preliminary. In the years since the review by Woodruff-Pak (1990), several studies have administered cognition-enhancing drugs such as memantine, galantamine, and donepezil (Woodruff-Pak et al., 2001, 2007b; Weible et al., 2004), as well as nefiracetam and physostigmine (Woodruff-Pak and Li, 1994; Woodruff-Pak et al., 2004) to older rabbits, and many of these drugs have been successful in ameliorating differences in EBCC performance. The rabbit version of EBCC engages both structures of interest (Criterion 7), since the cerebellum is essential and the hippocampus plays a modulatory role in the delay paradigm and is essential in the trace paradigm.

Though the rabbit version of EBCC satisfies the criteria identified in this review, the focus is on aging in the mouse (see **Table 4**). Given that the neural substrates of EBCC are conserved across many species, the test partially satisfies the fifth criterion of behavioral parallels across many species, though strain differences may contribute to differential results. In the case of C57BL/6 mice, age-related hearing loss may bias interpretation of results (Criteria 2 and 5). Age deficits have been reliably reported in other strains of mice that do not suffer from hearing loss, and both genetic and experimental lesions to the cerebellum in young mice cause deficits similar to those of older animals (Criteria 1 and 4). The shock US is relatively mild and no food restriction is required (Criterion 3). In contrast to rabbit studies, cognition-enhancing drugs have rarely been investigated in older mice (Criterion 6). Drug-induced deficits in EBCC can be rescued by the administration of nicotinic receptor agonists (Rodriguez-Moreno et al., 2006), but the effect of drugs on normal aged mice requires further exploration. Finally, EBCC in the mouse, as in the rabbit, cannot be considered a purely cerebellar task (Criterion 7).

FEAR CONDITIONING

Classical conditioning of fear is a paradigm that has received extensive study in recent years (**Figure 5**).

Neural substrates

The primary dependent measure of fear conditioning is freezing, which is normally defined as the absence of movement apart from respiration (Fanselow, 1990). A conditioned-fear response (freezing) can be elicited by either the originally neutral tone CS (cued fear) or the environment (context fear) in which the US was previously presented to the animal. This form of associative learning is rapid and robust, and the neural substrates have been fairly well defined, thus making this paradigm particularly attractive to researchers. Another benefit of fear conditioning is that it measures both hippocampus-dependent and -independent learning (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Rudy, 1993; Gerlai, 1998). Learning to associate the context with the US depends on the hippocampus while the association between the auditory CS and the US does not (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Gerlai, 1998). Both pharmacological (Logue et al., 1997a; Gerlai, 1998) and electrolytic (Kim and Fanselow, 1992; Phillips and LeDoux, 1992) lesions to the hippocampus result in a reduced amount of freezing to the context compared to non-lesioned controls.

The hippocampus is the essential site for contextual fear conditioning (Frankland et al., 1998; Maren et al., 1998). The ventral hippocampus may be the necessary substrate as excitotoxic lesions impair conditioning when administered to the ventral (Selden et al., 1991; Phillips and LeDoux, 1992) but not the dorsal hippocampus (Maren et al., 1997). Impaired context conditioning due to ventral hippocampus lesions may be the result of disrupting inputs to the nucleus accumbens, which would alter the exploratory behavior that is essential for normally exploring the environment (Maren, 1999), or by damaging the amygdala (Anagnostaras et al., 2001). Axon-sparing neurotoxic lesions may be the most appropriate because electrolytic lesions can damage fibers passing through the hippocampus (Maren, 1999). The other essential neural substrate required for cued fear conditioning is the amygdala, the site where the formation of the CS–US association occurs (Phillips and LeDoux, 1992).

Table 4 | Eyeblink classical conditioning: age sensitivity.

Study	Species/ strain	Age (months)	Age deficits (months)	Mirror lesions in young	Confounds/ strain differences	Effect of cognition-enhancing drugs	Cerebellum-sensitive	Hippocampus-sensitive
Brown et al. (2010)	*pcd* mutant	4–5		Loss of Purkinje cells impairs delay, not trace EBCC			Yes	
Kishimoto et al. (2001b)	PLCβ4 mutant	3–4		Loss of LTD in Purkinje cells impairs delay, not trace EBCC			Yes	
Kishimoto et al. (2001c)	C57BL/6	2, 11–12, 21–22	Delay: 21–22; Trace: 11–12+	Yes	Age-related hearing loss		Yes	Yes
Rodriguez-Moreno et al. (2006)	C57BL/6	3–5				Nicotinic agonist rescues drug-induced deficits		Yes
Vogel et al. (2002)	C57BL/6	4, 9, 12, 18	9+	Yes	Age-related hearing loss		Yes	
Weible et al. (2004)	Rabbit	2–3, 30–33	30—33	Yes		Galantamine rescues deficits		Yes
Woodruff-Pak (2006)	C57BL/6	4,8, 12, 18, 24	18+	Yes	Age-related hearing loss		Yes	
Woodruff-Pak et al. (2010)	CBA	4,8, 12, 18, 24	24	Yes			Yes	
Woodruff-Pak et al. (2007a,b)	Rabbit	26	Untreated rabbits impaired in acquisition	Yes		Galantamine improves acquisition		Yes

Species is mouse unless otherwise specified. C57 = C57BL/6.

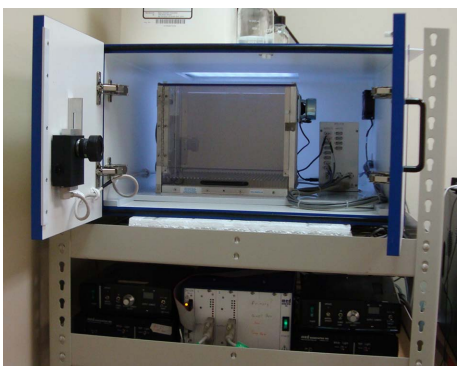

FIGURE 5 | Fear conditioning is a form of associative learning in which a previously neutral conditioned stimulus (CS), such as a tone, is paired with an aversive unconditioned stimulus (US), usually a foot shock. Through this pairing, the CS comes to elicit responses characteristically associated with the US.

At a neurotransmitter level, the cholinergic system has been implicated in context fear conditioning (Gould and Wehner, 1999; Gale et al., 2001; Feiro and Gould, 2005). Lesions of the nucleus basalis magnocellularis, which contains large cholinergic neurons that project to the neocortex, disrupts context but spares cued fear (Stoehr and Wenk, 1995). Scopolamine, a muscarinic cholinergic receptor (mAChR) antagonist, administered either systemically (Feiro and Gould, 2005) or directly into the dorsal hippocampus (Gale et al., 2001) impairs context but not cued conditioning. Administration of nicotine improves context learning while mecamylamine, a nicotinic cholinergic receptor (nAChR) antagonist, prevents the nicotine-induced improvements in context learning (Gould and Wehner, 1999). The administration of only mecamylamine did not alter contextual fear conditioning, which suggests that nAChR activation is not required for context learning but instead can serve a modulatory role. Additionally, nicotinic and muscarinic receptors interact to contribute to the learning that takes place in fear conditioning, as co-administration of nAChR and mAChR antagonists disrupts both cued and context fear learning, though the effects of this interaction may differ across the life span (Feiro and Gould, 2005).

Age deficits

Generally, aged rats and mice do not differ in the ability to freeze to an aversive stimulus, nor do they show deficits in the ability to form CS–US associations compared to younger animals (Doyere

et al., 2000; Gould and Feiro, 2005). One study reported that 22-month-old Wistar rats showed more freezing to the context than younger (7 month) animals, which may have been related to an increase in emotionality in the older rats (Doyere et al., 2000). More often reported is that older animals are not impaired on context fear, with the exception of long-term retention intervals of 20–60 days (Stoehr and Wenk, 1995; Oler and Markus, 1998; Houston et al., 1999; Mesches et al., 2004). Deficits in contextual fear have been reported at a 24-h retention interval for 11 month C57BL/6N compared to 8-month mice (Liu et al., 2003). Longer retention intervals also affect age-related abilities to retain cued fear information (Gould and Feiro, 2005).

In trace fear conditioning, a temporal gap exists between the presentation of the CS and the US. In this case, subsequent freezing to the tone CS requires the hippocampus to process the trace, or temporal gap, between the CS and US presentations (McEchron et al., 1998; Huerta et al., 2000; Villarreal et al., 2004). Older rats (22–24 month) are impaired in trace cued fear relative to younger (2–4 month) animals, whereas performance is equal for delay contextual fear conditioning (Villarreal et al., 2004). Contrasting studies with different strains of rats have shown no age differences in freezing among 2, 5, and 12 month animals in a trace fear conditioning paradigm (Cuppini et al., 2006). In mice tested at a 24-h retention interval, deficits in freezing to the context were seen in older (8 month) relative to younger animals (2, 4 month), whereas cued fear was not affected by age (Kaczorowski and Disterhoft, 2009). Thus, strain and paradigm differences can affect the ability of the fear conditioning task to detect age differences in both the trace and delay paradigms.

Age sensitivity

Of all the tests described in this paper, delay cued and contextual fear conditioning are perhaps the least sensitive to age. Whereas the Barnes maze or MWM show behavioral deficits in both the acquisition and retention of spatial memory, delay fear conditioning is acquired normally in rodents across the life span. Age deficits are only apparent at long retention intervals (48+ h). Decreases in neurogenesis in the dentate gyrus that accompany aging are not detected by fear conditioning, as no differences in cued or context freezing were seen in older rats tested on the trace paradigm even though they had reduced proliferation and new cell survival (Cuppini et al., 2006). Trace fear conditioning may be more sensitive to aging than delay fear conditioning as deficits in retention of cued and context fear are more consistently reported at earlier retest intervals.

Another potential drawback of the context fear paradigm is that electrolytic lesions to the hippocampus can increase motor activity, which invalidates the freezing response as a measure and makes interpretation difficult (Maren et al., 1998; Gale et al., 2001). Increased motor activity is not seen in pharmacological lesions of the hippocampus, so hyperactivity may not be a function of damage to the hippocampus but rather damage to the fibers of passage (Gale et al., 2001). In addition, Maren et al. (1998) reported no significant correlation between motor activity and context freezing in hippocampal lesioned rats, suggesting that a motor explanation for freezing deficits seems unlikely.

That lesions to the hippocampus differentially affect performance on the MWM and fear conditioning suggests that the two tasks require the hippocampus to a different extent. Since fear conditioning incorporates both a simpler dependent measure (freezing vs. motor control in swimming) and relies on more sensory systems (incorporating tactile, olfactory, and auditory stimuli into the context representation), it may be more difficult to see age-related differences in this task. In addition, context fear conditioning may be supported by other, non-hippocampal systems in certain situations (Owen et al., 1997; Gerlai, 1998; Gale et al., 2001). For example, lesions to the dorsal hippocampus prior to training reduce the amount of context freezing (Frankland et al., 1998; Gerlai, 1998) but do not abolish the ability to learn. Lesioned animals still show some freezing to the context, but the amount is reduced compared to sham lesioned controls. These results suggest a compensatory mechanism may be operating in the absence of the hippocampus that can allow the formation of the context–shock association. Normally, the hippocampus is involved in the formation of a unified, polymodal representation of cues in the training environment (Sutherland and Rudy, 1989; Frankland et al., 1998), which is then associated with the shock US (Phillips and LeDoux, 1992). Both rats and mice can represent the context through a single-cue strategy, which does not rely on the hippocampus and is normally suppressed (Logue et al., 1997a; Frankland et al., 1998). A single-cue strategy involves associating one or two salient cues of the context, rather than a unified representation, with the US shock. Context freezing is reduced compared to animals using a hippocampus-based strategy, suggesting that the non-hippocampal system may be less efficient, but still allow for learning.

With the exception of long-term retention, delay contextual fear conditioning does not generally reveal age-related performance deficits (see **Table 5**). Trace conditioning may be more sensitive to age differences (Criterion 1). Aged animals are able to perform the task, which suggests that the shock used is not too intense as a stressor (Criterion 3). There are several confounds that could account for differences in freezing to the context, including increased motor activity after electrolytic lesions to the hippocampus, strain differences and a secondary neural substrate that can support conditioning (Criteria 2 and 5). This alternate system that involves the peri-and postrhinal cortices precludes describing contextual fear conditioning as a purely hippocampal task (Criterion 7). In addition, the complicated results of lesion studies does not yield a unified behavioral response to compare to aged animals, though lesioned animals tend to show larger deficits than aged animals (Criterion 4). Since age deficits are not generally reported in contextual fear, the impact of cognition-enhancing drugs is undetermined (Criterion 6). Gould and Feiro (2005) reported that galantamine administered to aged C57BL/6 mice reversed deficits in retention of cued fear, whereas no deficits were reported in context fear. Thus, contextual fear conditioning does not appear to satisfy any of the criteria to evaluate age-sensitivity. A modification of the context fear paradigm, the context pre-exposure facilitation effect (CPFE), affords increased sensitivity to hippocampal disruption and the potential application to aging studies.

CONTEXT PRE-EXPOSURE FACILITATION EFFECT

Neural substrates

When a rodent is placed into a context and a shock is delivered within a short amount of time (2–3 s) the animal will show little to no fear to the context when tested later (Blanchard et al.,

Table 5 | Fear conditioning: age sensitivity.

Study	Species/ strain	Age (months)	Age deficits	Mirror lesions in young	Confounds/ strain differences	Effect of cognition-enhancing drugs	Cerebellum-sensitive	Hippocampus-sensitive
Cuppini et al. (2006)	Sprague-Dawley rat	2, 5, 12	None	No				No
Doyere et al. (2000)	Wistar rat	7, 22	22 months froze more to context	No				No
Feiro and Gould (2005)	C57BL/6	2–3, 19–20	19–20 months to CS at 48+ h retention	No				Yes
Gerlai (1998)	C57BL/6, DBA/2	3–4	Not rested	Lesions partially impair	DBA more sensitive to shock; C57 froze more to context and tone CS			No
Gould and Feiro (2005)	C57BL/6	2–3, 19–20	19–20 to CS at 48+ h retention	No		Galantamine-rescued deficits		No
Gould and Wehner (1999)	C57BL/6	2–3				Nicotine enhances context learning		Yes
Houston et al. (1999)	Fisher 344 rat	3, 9, 27	27 months to context in 20–60 day retention	Yes				Yes
Kaczorowski and Disterhoft (2009)	C57/SJL F1 hybrid	2–4, 8	Subset of aged impaired on context	Yes				Yes
Liu et al. (2003)	C57BL/6	8, 11	11 months to context in 24-h retention	Yes				Yes
Mesches et al. (2004)	Fisher 344 rat	6, 18	18 months to context in 48-h retention	Yes				Yes
Oler and Markus (1998)	Fisher 344 rat	4, 10, 23	23 months to context in 52-day retention	Yes				Yes
Owen et al. (1997)	12 inbred and seven hybrid strains	2–3			Good: C57, 129, hybrids; Poor: FVB, DBA, Bub, C3H			
Paylor et al. (1994)	C57BL/6, DBA/2	3–4			C57 froze more to context			Yes
Stoehr and Wenk (1995)	Fisher 344 rat	3, 9, 24	24 months	Yes				Yes
Villarreal et al. (2004)	Fisher 344 rat	2–4, 22–24	22–24 months impaired on trace cued	Yes	No age deficits in delay paradigm			Yes

Species is mouse unless otherwise specified. C57 = C57BL/6.

1976; Fanselow, 1990). This phenomenon is called the immediate shock deficit, and Fanselow (1986, 1990) suggested that the deficit is caused by a failure of the animal to associate the contextual stimuli present at the time of the shock with the shock. Increasing the placement to shock interval with delays up to 2-min increases the amount of freezing to the context. In addition, allowing the rat to explore the conditioning chamber the day before the immediate shock increases the amount of freezing to the context. This increased exposure and resulting contextual fear is termed the CPFE. The pre-exposure establishes a representation of the context that can be retrieved by a brief exposure to the context prior to shock presentation. This effect has been reported primarily in rats (Kiernan and Westbrook, 1993; Rudy and O'Reilly, 1999; Matus-Amat et al., 2004; Pham et al., 2005) but also in mice (Wiltgen et al., 2001; Frankland et al., 2004; McHugh and Tonegawa, 2007; Kenney and Gould, 2008; Goeldner et al., 2009).

Subsequent research has shown that the immediate shock deficit depends on the formation of a representation of the context and not on handling manipulations, novelty of the conditioning chamber (Landeira-Fernandez et al., 1995) or processing of the foot shock US (Landeira-Fernandez et al., 2006). An essential component of the context representation requires spatial exploration of the cues in the environment (McHugh and Tonegawa, 2007). Context-specific place cells in the CA1, CA3, and dentate gyrus subfields of the hippocampus provide spatial information to which the shock is associated. It has also been shown that rats condition to the pre-exposure environment and not the shock environment. If the pre-exposure context is different from the shock context, rats will show freezing when tested in the pre-exposure but not the shock context (Rudy and O'Reilly, 2001).

A potential explanation for the conflicting results of lesion and aging contextual fear conditioning studies is that conditioning can be supported by two distinct neural systems (Rudy et al., 2002, 2004; Rudy, 2009). The features of a context can be bound together into a conjunctive representation, and it is this unified, polymodal representation that becomes associated with the shock (Rudy and O'Reilly, 1999; O'Reilly and Rudy, 2001). A second system involves an elemental or feature representation of the context, whereby certain salient features become associated with the shock (Frankland et al., 1998). The conjunctive representation is supported by the hippocampus, whereas the elemental representation is supported by a neocortical system including the peri- and post-rhinal cortices (Rudy, 2009). The application of the CPFE paradigm has confirmed that the hippocampal system is indeed responsible for the conjunctive representation of the context and that elemental conditioning does not occur. Rats pre-exposed to the conditioning context but not those exposed to its individual features showed the CPFE (Rudy and O'Reilly, 1999). Neurotoxic lesions to the dorsal hippocampus before pre-exposure prevented the CPFE, and the same lesions did not affect standard context fear (Rudy et al., 2002). Additionally, pharmacologically inactivating either the neurons (Matus-Amat et al., 2004) or NMDA receptors (Matus-Amat et al., 2007) in the hippocampus prevented the CPFE but had no effect on standard context fear. Thus, in normal, intact rats, the role of the hippocampus is to create and store a conjunctive representation of the context as well as to inhibit elemental conditioning to individual features of the context. That standard context conditioning can occur in the absence of a functional hippocampus suggests that the secondary (neocortical) system is able to support the task through elemental associations (Rudy et al., 2002, 2004; Matus-Amat et al., 2004, 2007; Rudy, 2009).

The power of the CPFE paradigm is that it isolates the hippocampus-dependent portion of the fear conditioning task (conjunctive representation of the context) from the context–shock association. This paradigm guarantees a focus on the hippocampus, and a number of studies have used this task to assess the specific contribution to cellular and molecular components of learning. Kenney and Gould (2008) reported that nicotine enhances context learning, but not the formation of the context–shock association. Other studies have implicated consolidation in the hippocampus as an essential component of the CPFE (Barrientos et al., 2002a,b; Frankland et al., 2004). Inhibiting protein synthesis in the dorsal hippocampus (thereby preventing consolidation of the conjunctive context representation) after pre-exposure led to a reduced CPFE (Barrientos et al., 2002a). A similar result was reported when the inflammatory cytokine IL-1β was injected after pre-exposure, though it also showed time-bound effects in that CPFE was not inhibited when the compound was injected 48 h after pre-exposure (Barrientos et al., 2002b).

Thus it seems that the hippocampus (1) rapidly acquires a conjunctive representation of the context upon pre-exposure, (2) stores this context representation, and (3) is needed for the activation of the context representation to be associated with the US shock (Matus-Amat et al., 2004; Matus-Amat et al., 2007; Rudy, 2009). In addition, the memory of the context can be activated by a subset of the original cues, which is referred to as pattern completion (Rudy and O'Reilly, 1999, 2001). Rudy et al. (2002) and Barrientos et al. (2002a,b) have developed a modification of the CPFE protocol that establishes an association between the contextual representation and the transport cues preceding the placement of the rat into the context. The method involves multiple exposures to the context in which the rat is transported in a distinct light-sealed black container. Thus, on shock and test days, the transport cues can activate the representation of the context. In this method the rat can be shocked immediately when placed in the chamber and still show robust context fear on test day, which eliminates the need for pattern completion to take place in the chamber on shock day as originally suggested by Fanselow (1990).

Age deficits and age sensitivity

To date, no study has incorporated the CPFE to examine changes in hippocampal function across the life span. There are a number of studies demonstrating that disruption of the hippocampus impairs the CPFE while sparing standard context fear (see Rudy et al., 2004; Rudy, 2009 for a review). It seems plausible that changes in synaptic efficiency and plasticity, which are thought to support age-related changes in behavior on tests of learning and memory, could act as "disruptions" comparable to the pharmacological and electrolytic lesions commonly used in the CPFE literature. It remains to be seen if aging in the hippocampus causes deficits in CPFE, but the paradigm seems ideal for these studies as it isolates the hippocampus-dependent aspects of context fear and is not confounded by the possibility of other neural systems maintaining the behavior. Sensitivity to hippocampus insult is certainly greater in

Table 6 | Context pre-exposure facilitation effect: age sensitivity.

Study	Species/ strain	Age (months)	Age deficits	Mirror lesions in young	Confounds/strain differences	Effect of cognition-enhancing drugs	Cerebellum-sensitive	Hippocampus-sensitive
Frankland et al. (1998)	C57BL/6	3–5		Lesions impair				Yes
Kenney and Gould (2008)	C57BL/6	2–3				Nicotine enhances context learning		Yes
Matus-Amat et al. (2004)	Long–Evans rat			Lesions impair				Yes
Rudy et al. (2002)	Long–Evans rat			Lesions impair				Yes

Species is mouse unless otherwise specified. C57 = C57BL/6.

the CPFE compared to standard contextual fear conditioning and theoretically this difference should make the CPFE more sensitive to processes of aging.

Based on the current CPFE literature, this test only satisfies the seventh Criterion – it seems to be a purely hippocampal task (see **Table 6**). Whereas standard contextual fear conditioning can be supported by a non-hippocampal neural system, the CPFE has no alternative neural substrates. Criterion 4 is also partially supported, in that young animals with lesions to the hippocampus show CPFE deficits expected in older animals. No study has investigated the CPFE in aged animals, so this criterion requires further exploration. No data exist to address the remaining five criteria and future studies are needed to determine the age sensitivity of the CPFE.

CONCLUSION

The ideal age-sensitive behavioral test in mice should:

1. Reliably discriminate between young and old animals on the target behavior
2. Avoid confounds from other processes (i.e., visuomotor in MWM, non-hippocampal system in context fear)
3. Avoid food restriction or other stressors that could weaken older animals
4. Mirror deficits of young lesioned animals in an aged population
5. Identify the differences in strain and show parallels across species
6. Reveal improved performance when animals are treated with drugs known to enhance behavior in the aged in clinical trials
7. Engage neural substrates that support learning and memory (hippocampus and/or cerebellum)

The behavioral tests discussed in this review have been implemented with varying amounts of success to assess normal aging in mice. To increase age sensitivity in cases where known strain differences exist (rotorod), multiple strains of rodent should be tested at multiple ages to account for different baseline performance combined with differential trajectories of ability across the life span. Differences become especially important when strains that provide a genetic background to models of degenerative disease and accelerated aging are involved. To account for heterogeneity of aging, particular attention should be paid to recognizing subpopulations of good and poor performing animals (MWM, EBCC). In addition, genetically heterogeneous mice created by a four-way cross (Sumien et al., 2006) are an attractive possibility that would lead to wider generalizability of age-related declines in cognitive performance across the life span compared to genetically uniform inbred strains most often utilized in rodent models of aging.

To improve age sensitivity in the MWM, manipulations to increase the task demand (repeated acquisition, working-memory versions) should be implemented. Dependent measures that are separate from motor skill biases such as platform crossings and distance traveled should be considered, while an emphasis solely on latency to escape should be avoided. If mice and rats perform differently on a particular task (Barnes maze), specific dependent measures should be included (primary path length, primary latency) to account for these differences. In the case of contextual fear conditioning, which does not appear to be age sensitive, methodologies that do not allow for alternate neural systems to support the behavior (such as the CPFE) should be applied to assess the contribution of the hippocampus to this task across the life span. Comparisons between multiple behavioral tests that differentially engage the structures of interest (i.e., fear conditioning, MWM and Barnes maze for the hippocampus) and those that have very well elaborated neural substrates and wide applicability to a number of species (EBCC) would yield the strongest conclusions.

In particular, two of the behavioral tests discussed here have been implemented in little (Barnes maze) to no (CPFE) studies of aging in the mouse, despite the fact that both show exceptional promise. A robust data set for the Barnes maze has been compiled in the rat, but no studies have evaluated the CPFE across the multiple ages in rodents. The data do not yet exist to draw satisfactory conclusions on the age sensitivity of these two tasks with respect to aging in mice. Studies assessing the performance of multiple strains (or heterogeneous mice) across the life span are needed.

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