Which Comes First, “I forgot!” or “What did you say?”: Identification of Cognitive and Sensory Decline in Aging Animals

Lauren Anderson
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Advisor: Prof. Alev Erisir
Second Reader: Prof. Ching-Ling Teng
Abstract

Understanding why neurons are not all equally susceptible to aging has important significance for future research concerning aging in general and neural degenerative disorders. In order to answer the question of whether brain regions age at comparable rates within individuals, adult rats were run through memory and sensory tests twice over a one-year period. There was no positive correlation between the rates of decline in the two behavioral tests, but each test had a majority of the animals show a decline in functioning, suggesting decline rate differs not only by brain area but also by subject. It’s no secret that aging affects our bodies and our brains, but not all aging is created equal. Not even all parts of the brain decline at the same rate.
Which Comes First, “I forgot!” or “What did you say?”; Identification of Cognitive and Sensory Decline in Aging Animals

It’s no secret that aging is inevitable, we all know as we grow older our bodies and minds will experience the effects of time. The brain is no exception to this; the human brain experiences a period of growth and then decline as we move through the life cycle. One major aspect of brain aging is a decrease in volume; in adults ages 71-80 brain volumes were found to have decreased significantly in size when compared to middle aged adults (Courchesne et al., 2000). Gray matter, which contains the neuronal cells and which increases quickly during developmental years and gradually plateaus in adulthood, decreases gradually with normal aging (Courchesne et al., 2000). The loss of white matter, or myelinated axons, is often associated with Alzheimer’s, but a general decline in myelinated fibers has also been shown in healthy aging adults (Tang, Nyengaard, Pakkenberg, & Gundersen, 1997).

Changes in neural density and brain tissue volume have been shown to be correlated directly with aging and decline (Raz et al., 2005). Taking a step further and looking into the aging of circuits and individual neurons has shown aging affects certain synapses and circuits, disrupting functions like memory (Morrison & Hof, 2002). This suggests that the aging of brain areas is not merely a decrease in volume or a decrease in neurons overall, but more so a change of individual neurons within a circuit (Duan, Wearne, Rocher, Macedo, Morrison, & Hof, 2003). Measuring dendritic spine changes, specifically dendritic spine number and density has been shown to be a reliable measure of neural decline (Duan, Wearne, Rocher, Macedo, Morrison, & Hof, 2003). Dendrites’ spine numbers and density decreased due to aging (Duan, Wearne, Rocher, Macedo,
Morrison, & Hof, 2003) and these changes have been correlated with behavioral age related impairments, such as memory and cognitive function loss (Peters, Sethares, & Moss, 1998b).

Certain brain areas have been found to be more susceptible to aging, as particular regions have shown more rapid neural decline (Hedden & Gabrieli, 2004). Cross-sectional studies using correlations between age groups have shown more neural reduction in prefrontal gray matter when compared to sensory cortices (Gunning-Dixon & Raz, 2000), and longitudinal studies have shown that changes in brain volume are widespread but not equal in every area (Raz et al., 2005). It has even been demonstrated that separate layers of a section of cortical neurons show different levels of decline (Duan, Wearne, Rocher, Macedo, Morrison, & Hof, 2003).

Alongside quantitative measures, behavioral measures of aging have also shown that different parts of the brain age differently. The effects of aging on different types of memory are a great example of this. Performance-based tasks, such as remembering how to ride a bike or remembering to how to perform an action when asked, have been shown to be resistant to aging whilst cognitive-based tasks, such as remembering a phone number or a list of words, are notably susceptible (Churchill, Stanis, Press, Kushelev, & Greenough, 2003). This suggests that even though they are both cognitive processes of memory where the subject is actively trying to remember, time does not affect the two circuits equally.

Decline of sensory ability due to aging is harder to study since the mechanical aspects of sensory reception are also susceptible to aging. It has been suggested that sensory decline may be more due to the breakdown of the sensory mechanisms than
neural degeneration (Caspary, 2010). However, an examination of aging of the olfactory circuit suggested that different parts of the circuit, particularly afferent synaptic input, were more prone to aging than the entire olfactory bulb circuit, as the majority of the OB layers did not change volume throughout aging (Richard, Taylor, & Greer, 2010). This suggests that while some sensory loss, for example hearing loss, may be due to mechanical problems, in this example with the ear, there is also change on a neural level. In a study looking at the cellular and subcellular changes within the visual and auditory cortices in mice, it was found that there were definitely cellular changes that contributed to the loss of sensitivity in hearing and vision (Tremblay, Zettel, Ison, Allen, & Majewska, 2012).

It has been demonstrated that neurons are not all equally susceptible to aging through both behavioral and neural anatomical measures, so it is possible that the parts of the brain that handle cognition and system information will not have an equal change over time. All parts of the brain are going to change, that is decrease in volume, to some extent (Raz et al., 2005), and it is know that certain parts of the brain are going to decrease at a more rapid rate. By using behavioral tests of cognition and audition, the aim is to investigate the effect of aging, by looking at amount of behavioral decline, on these two functions within individual subjects.

In order to look at changes due to aging, a within subjects longitudinal design is necessary. It is necessary to look at the same group of rats because if you were to compare abilities of different age rats you wouldn’t have a true aging study because you would be looking at averages across groups, not changes due specifically to aging. By using the same subject group of animals over a year period, changes in behavior can be
attributed fairly confidently to aging. A longitudinal design is necessary to capture the aging effect itself, testing subjects more than once across a time period allows for a direct measure of change in ability over time.

Because the Radial Arm Maze (RAM) is a tried and true measure of cognitive ability, and includes a circuit that is affected by aging, it was selected as the cognitive measure in this experiment (Chrobak, Hanin, Lorens, & Napier, 1995; Kolata & Kolata, 2009). For a reliable measure of sensory ability, the acoustic startle response (ASR) is an easy to obtain and reliable measure (Koch, 1999).

By using a within subjects longitudinal study that looks at the cognitive and sensory abilities of rats, we aimed to gain more of an understanding of the relationship between these two brain functions and their susceptibility to aging. The aim is to investigate if brain areas age at comparable rates within individuals and to see if there is a relationship between the decline of the two behavioral functions. It is hypothesized that for all of the subjects there will be a decline in cognitive and sensory ability, but that the extent of this decline will vary by animal. Additionally, it is thought the decline of cognition and sensory ability will not be correlated when compared between animals. Gaining an understanding about how the normal healthy brain ages will not only help us combat the effects of time on our minds but also will further our understanding of degenerative diseases if we are able to classify and understand normal neural decline.

**Method**

**Animals**

Five male Sprague Dawley rats, starting at PM 7 and PM 11, were tested twice using the RAM and an ASR test (see Table 1). There was a 5-month period between the
RAM testing (at PM 12 and PM 17 and then at PM 17 and PM 22). There was a 10-month period between Startle Box testing (at PM 7 and PM 11 and then at PM 17 and PM 22). Rats were individually housed on a 12:12 light: dark cycle, and behavioral testing was performed during the dark phase of the cycle. All animal procedures were approved by the University of Virginia Animal Care and Use Committee.

**Radial Arm Maze**

Cognitive ability was measured using a standard 8-arm radial arm maze (See Figure 1). The maze, elevated off the ground to prevent the rats from escaping, had clear pieces of Plexiglas to block entrance to the arms when necessary. A depression at the end of each arm allowed for baiting without the rat being able to see the pellet until it had reached the end. Food deprivation of the rats to 85% of their free-feeding body weight was necessary to ensure motivation within the maze. Pre-exposure to the rodent chow pellets while in the home cages during daily feeding habituated the rats to the pellets used as rewards in the maze.

For the initial training, the rats were put into the middle of the maze with four baited and four blocked arms and were given five minutes to retrieve all four pellets. Animals were removed from the maze when they successfully visited all four arms or the five minutes were up. An error was defined as an entry into an unbaited arm, in this case an arm in which the rat had already entered. This training was performed until the rats had a stable average error rate over several days (See Table 2).

After single trial training, the rats were put on win-shift training. Initially, four randomly selected arms were baited and open. The rats were once again given five minutes to visit all four arms. After completion of the first phase, the rats were removed
and the maze cleaned. After a 2-minute delay, the rats were placed in the maze with all of the arms opened, with the four previously unbaited arms now baited. The rats were trained to visit the four baited arms with as little error as possible. Error is defined as re-entry into an arm that had been baited in the first phase or an arm that had been visited before during the second phase. After training to asymptote of learning, the rats were tested for data collection for 14 days each testing period.

**Startle Box**

Sensory ability was measured using the startle response to tones. The system provides a quantifiable response to stimuli by measuring the rat’s muscle freeze reaction. When rats hear a loud sound, they instinctively freeze. Depending on how loud the sound is and how shocked the rat is, they freeze more or less. This difference can be measured and viewed as ability to hear and process sensory information. A SR-LAB system (San Diego Instruments) was used, which contains a chamber in which a Plexiglas cylinder is attached to an accelerometer. The rats experienced tones varying from 65 dB (baseline) up to 110 dB at differing lengths and intervals to avoid habituation. The rat’s movement in response to the sounds of varying decibels was recorded.

**Results**

**Radial Arm Maze**

Performance in the Radial Arm Maze was compared using Percent Error, \((\text{Number of Errors})/\text{(Total Number of Arms Visited)}\)*100. Average performance was calculated for each of the testing periods. The results are available in Table 3. Four out of five animals displayed a reduction in their performance (Figure 2). The range of decline was 13.30% to 1.70% (excluding the animal which showed a 8.54% improvement). The slope of decline was calculated, \((\text{Performance at B} - \text{Performance at A})/ (\text{Age at B}- \text{Age at A})\),
and graphed (Figure 3). These results suggest that the majority of the animals showed a decline in memory ability, but there was a large amount of variance in the amount of decline.

**Auditory Startle Test**

Performance in the Startle Box was calculated as the point of inflection on the startle curve. Rats’ startle response increases as a polynomial function over increasing decibels, with the inflection point of the curve resulting in a reliable measure of startle response (Koch, 1999). Fitting the data from 65 to 110 decibels to a curve, and finding the second derivative of that curve calculated the point of inflection of startle of each rat. This allowed for a single measure for each rat’s startle response. The results are available in Table 3. Four out of five animals showed a reduction in performance, while one animal showed little change in its startle response (Figure 4).

The slope of decline was calculated for auditory performance (Performance at B – Performance at A)/ (Age at B- Age at A), and graphed (Figure 5). Once again the majority of the animals showed a decline in performance, but there was a lot of variance in the amount of decline.

**Cognitive vs. Auditory**

In order to compare the decline of cognitive and auditory ability, the slope of declines were compared (Figure 6). The animal that did not show a decline in the RAM is not the same animal that did not show a decline in the startle box, suggesting there is not comparable aging between the two behavioral systems. There was a negative correlation for Startle Slope verses Radial Arm Maze Slope, $r (3)=-.893$, $p = .041$ (Figure 7). The
fact there is a negative correlation once again suggests there is not comparable aging between the two systems.

**Discussion**

These experiments demonstrated that both REM and ASR revealed behavioral decline in most animals tested. Out of the five animals tested between PM 7 and PM 22, one animal improved in the cognitive measure, one animal did not change in the sensory measure, and the rest showed a decline in both measures, suggesting that there may be life-long deterioration in cognitive and sensory functions in most subjects. When present, the amount of decline for each measure varied greatly across the animals, indicating individual susceptibilities for the time course and the severity of the decline. The severity of decline between cognitive and sensory measures did not positively correlate, suggesting that age-dependent function loss is not a uniform feature of all neural functions. Thus our results do not provide evidence that cognitive functions decline earlier than sensory or vice versa, at least at the ages that are examined. Regardless, the capacity for REM and ASR paradigms to reveal decline in cognitive and sensory abilities indicates these paradigms can be used in identification of individual animals with dementia in correlative studies of psychophysics and neuroanatomy.

We had initially hypothesized that behavioral functions that particularly rely on different brain regions would age differently. We used the RAM paradigm to measure the short-term memory function of the rats since the RAM is a very commonly used measure of memory, particularly working memory (Kolata & Kolata, 2009). Aging has been shown many times to have a negative affect on performance in the RAM (Shukitt-Hale, McEwen, Szprengiel, & Joseph, 2004) and this deficit has been shown to be a result of
memory dysfunction and not general performance decline (Chrobak, Hanin, Lorens, & Napier, 1995). Studies have shown that mistakes made by older rats are due to interference from choices, not limited time (Olton & Samuelson, 1976). Using this paradigm, it was shown previously that rats show a decline by PM 22 (Barnes, 1987). The type of short term-memory measured here relies on normal function of the posterior parietal cortex (Dimattia & Kesner, 1988) and any deterioration in function would indicate deterioration in neural function in these areas. Our results indeed indicated that short-term memory declines between PM 12 and PM 23. Shukitt-Hale et al. (2004) found by PM 21 rats were showing significant decline in the RAM. However our study looked at the decline of the same rats over time while the Shukitt-Hale et al. (2004) experiment compared younger rats to older rats.

We used the ASR paradigm for sensory behavior since the startle response is an instinctual response that does not need to be learned in order for the animal to perform. A rat instinctively freezes when it hears loud sounds, and the physical freezing is mediated at the brainstem and does not include upper cognitive processes (Swerdlow & Geyer, 1999). Since the actual physical startle response is not affected by age (Krauter, Wallace, & Campbell, 1981), it is a reliable measure of neural auditory ability for aging. As the rat becomes less able to process the sound, it freezes less. By randomizing the time period between sounds, and randomizing what decibel level the rat is exposed to, on a range from below baseline 65 dB to 110 dB, habituation was prevented. Our results indicated that auditory ability declines between PM 7 and PM 21. The fact that we found auditory decline at this age is notable, as previous research into auditory decline has illustrated
decline at much older ages, closer to PM 23 (Mendelson & Ricketts, 2000) and PM 25 (Turner, Hughes, & Caspary, 2005).

Our results also suggest that the brain does not age uniformly, and which function will deteriorate first may be dependent on the individual. Decline in one measure did not mean there would be a comparable decline, or even a decline at all, in the other measure; it depended on the animal. One animal showed a decline in cognitive measures but did not show a change in sensory functioning. One animal that showed the most decline in sensory functioning improved its performance in the RAM. Three of the animals showed a decline in both, but there was a large variety in amount of decline for both sensory and cognitive abilities. If the brain aged equally for each animal, the relationship between the slopes of decline of the two measures would be positive. Instead the negative correlation suggests aging differs by area within each individual. Previous studies comparing cognitive ability to visual sensory ability have shown a very weak correlation between sensory and cognitive ability in aged adults (Lindenberger & Ghisletta, 2009), so these results further support the idea that brain regions do not age at comparable rates within an individual.

There was a large variance in the amount of decline for both measures, meaning there were not similar decline rates for sensory or cognitive ability. The fact there was not a general trend for either behavioral measure suggests that even within a certain brain functions, it is very individual as to how much it will be affected by age. The majority of animals showed decline in both behavioral tests, but both tests had one animal that did not decline. Previous studies using the RAM and old rats showed the more serious decline happens around PM 22-24, so the fact that the majority of the rats showed a
measurable decline before this point is indicative of the sensitivity of our experimental setup (Barnes, 1987). Auditory decline in rats has also been shown to be significant at PM 20 (Swetter, Fitch, & Markus, 2010), so once again finding a measurable decline in the majority of the rats who were younger than this at most of the testing times suggests notable decline happens even during middle age. The idea of using a within subjects design in an aging project to compare different behaviors is somewhat novel, the majority of the current published aging work had used the technique of comparing old to young subject groups, or looking at only one behavioral change over time. By looking at the same rats as they aged we were able to look at how individuals change, and didn’t have to simply compare average decline rates.

As we continue to understand more about how the brain and neurons age, it is important to continue to understand the effect of aging on behavior. It is known that neurons lose dendritic spines, along with excitatory synapses formed on them as the brain ages (Duan, Wearne, Rocher, Macedo, Morrison, & Hof, 2003), however how these synaptic changes correlate with behavioral decline is yet to be elucidated. Concurrent behavioral and neuroanatomical studies are in a good position to reveal the neural bases of behavioral decline in the aging brain, as well as allowing the design of new studies that aim to delay the neuronal decline. To further investigate the question of whether brain areas age at comparable rates, the next step in this study is to use behavioral techniques and additionally neural measurements of aging. By using dendritic density and spine number as a measure of neural decline (Duan, Wearne, Rocher, Macedo, Morrison, & Hof, 2003), it will be possible to investigate whether there is a relationship between behavioral decline and neural decline. In order to compare behavior and neural changes it
is necessary to run behavioral tests at least twice to get a measure of decline and then compare those animals’ dendritic density at the end of testing. By getting samples of different ages as the study progresses, it will be possible to compare the different densities with differences in behavioral ability.

The primary aim of the current study was to establish a reliable approach to characterize the amount of decline in individual animals, which would further be tested using anatomical approaches. The design of the experiments, testing each animal at two different behavioral tests at two ages, allowed a second aim to be addressed, namely testing our hypotheses regarding how the time course of cognitive and sensory functions correlate. There was a particular shortcoming for this second aim and that was the small sample size. The small number of animals meant a limited amount of data, so statistically significant results comparing population of animals were difficult to obtain. However, even with the small numbers the trend of differences in aging between brain regions was visible. Increasing the number of animals in a future experiment would improve the results, but having more than two testing periods would give even more information. A study design where the animals were tested four times over two years, for example, would allow for revealing the time courses of cognitive and sensory declines with better precision and allow their statistical comparisons.

The current study took a new step in the field of aging research by comparing behaviors in the same animals as they aged. The novelty in the study design allowed for conclusions about changes over time in individuals without looking at averages for age groups or focusing only on one ability. This allowed for a wider picture of aging, one that shows how varied aging can truly be. For future aging research, it might be necessary to
not just simply look at the average rates of aging for certain brain functions but also understand aging on a more individual level. An understanding of what is happening on a neural level would take some of the aging guesswork away. Knowing what behavioral changes happen and how neurons change as a brain ages is much more important than learning what the average decline across a population is. It is important to know averages and ideas about the entire population, but when it comes to treating or helping someone that is aging, it is important to remember their brain is unique. The current study and any future research are important for both neural degenerative disorder research and for understanding aging in healthy adults. Understanding how a healthy adult ages, and what will happen to their brain, will help us in the future with aiding the elderly.
References


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doi:10.1038/nrn1323


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Table 1

*Age of Each Animal at Testing Points.*

<table>
<thead>
<tr>
<th>Animal</th>
<th>Age RAM Test 1 (PM)</th>
<th>Age RAM Test 2 (PM)</th>
<th>Age Startle Test 1 (PM)</th>
<th>Age Startle Test 2 (PM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C07</td>
<td>12</td>
<td>18</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>C09</td>
<td>12</td>
<td>18</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>C11</td>
<td>12</td>
<td>18</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>E01</td>
<td>17</td>
<td>23</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>E04</td>
<td>17</td>
<td>23</td>
<td>11</td>
<td>22</td>
</tr>
</tbody>
</table>
Table 2

*Radial Arm Maze Training Schedule.*

<table>
<thead>
<tr>
<th>Event</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food Deprivation and Habituation</td>
<td>D1-D7</td>
</tr>
<tr>
<td>- Until 85% of body weight and comfortable in the maze</td>
<td></td>
</tr>
<tr>
<td>Phase 1 Training</td>
<td>D8-D14</td>
</tr>
<tr>
<td>- Until able to complete visiting 4 arms in less than 5 minutes</td>
<td></td>
</tr>
<tr>
<td>Phase 2 Training</td>
<td>D15-D21</td>
</tr>
<tr>
<td>- Until error rate for Phase 2 has reached asymptote</td>
<td></td>
</tr>
<tr>
<td>Data Collection</td>
<td>D22-D36</td>
</tr>
<tr>
<td>- 14 days worth of RAM Win-Shift training</td>
<td></td>
</tr>
</tbody>
</table>
Table 3

Results of average performance in each of the behavioral tests by animal.

<table>
<thead>
<tr>
<th>Animal</th>
<th>RAM Test1 (Percent Error)</th>
<th>RAM Test 2 (Percent Error)</th>
<th>Startle Test 1 (Point of Inflection)</th>
<th>Startle Test 2 (Point of Inflection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C07</td>
<td>22.822</td>
<td>14.285</td>
<td>58.631</td>
<td>82.848</td>
</tr>
<tr>
<td>C09</td>
<td>18.299</td>
<td>29.852</td>
<td>73.630</td>
<td>83.579</td>
</tr>
<tr>
<td>C11</td>
<td>25.396</td>
<td>27.096</td>
<td>72.779</td>
<td>93.812</td>
</tr>
<tr>
<td>E01</td>
<td>44.002</td>
<td>51.175</td>
<td>72.036</td>
<td>78.124</td>
</tr>
<tr>
<td>E04</td>
<td>46.857</td>
<td>60.158</td>
<td>90.406</td>
<td>90.338</td>
</tr>
</tbody>
</table>
Figure 1. Radial Arm Maze Design

Arm Length: 60 cm
Arm Width: 9.5 cm
Central platform Diameter: 39 cm
Platform height: 60 cm
Food cups diameter: 2 cm
Food cups depth: .5 cm
Figure 2. Decline by animal in the Radial Arm Maze.
Figure 3. Slope of Decline in the Radial Arm Maze.
Figure 4. Decline by Animal in the Startle Box.
Figure 5. Slope of Decline in the Startle Box.
Figure 6. Comparison of Decline by Animal in the Radial Arm Maze and the Startle Box.
Figure 7. Correlation of Slope of Startle Box Decline Compared to Radial Arm Maze Decline.