Differential aging-related changes of D1, D2, and D3 dopamine receptor expression in the striatum

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I hereby declare I am the sole author of this thesis. It is the result of my own work and is not the outcome of work done in collaboration, nor has it been submitted elsewhere as coursework for this or another degree.

Signed:_____ Date:_____

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ABSTRACT - Aging is associated with a decrease in motor function and a concomitant increase in muscle stiffness and tone. The striatum plays a critical role in the control of motor function, and it receives strong dopamine (DA) innervation from the substantia nigra. DA actions are mediated by both excitatory D1-like (D1 and D5) and inhibitory D2-like (D2, D3, and D4) receptors, and D1, D2, and D3 receptor subtypes are thought to be involved in motor control, however there is a lack of data on aging-related DA receptor expression levels in the striatum. We hypothesize that the observed behavioral aging-related changes in motor control might be associated with a change in striatal DA receptor with age, possibly via a shift in inhibitory/excitatory DA receptor expression. Three groups of mice (C57BL/6) aged 2 months (n=4), 1 year (n=4), and 2 years (n=4) were used in this study. Striatal tissue was removed from the left hemisphere and Western blots were performed, to detect DA receptors D1, D2, and D3 expression levels (Abcam, D1: ab78021; D2: ab21218, D3: ab42114). DA receptor expression levels were normalized to ß-actin and the respective DA receptor expression in 2-month old animals. We found that with age, D1 receptor expression increased continuously and significantly over a ~4 fold increase (383.2 ±62.4 %) in the 1 year old and reached a ~5 fold increase (474± 49.5 %) in the 2 year old animals ($p<0.001$). In contrast, D2 receptor expression did not change with age (1 year: 110.8 ±2.81 %; 2 year: 121.0 ± 17.0 %, $p = 0.556$). Similarly, D3 receptor expression showed no change with age (1

year: 147.1 ± 6.83 %; 2 year: 122.1 ± 11.4 %, p = 0.078). Together these data indicate an increase in excitatory striatal DA receptor expression levels with age. Our data suggest that this net excitatory increase may play a role in the decline in motor function with age. It is tempting to speculate that the increase in D1 receptor expression might be a homeostatic compensation for the well-established reduction of DA levels with age.

Acknowledgments

I would sincerely like to thank the people and organizations that made this study possible: My parents, the Clemens lab, the Department of Biology, East Carolina University and the Department of Physiology, Brody School of Medicine. I would also like to thank my secondary advisor, Dr. David Rudel, for his continued support throughout my university career and beyond. I am especially grateful to my project advisors, Dr. Stefan Clemens and Dr. Ben Keeler, for their knowledge and guidance. I cannot emphasize enough how grateful I am to Dr. Clemens for sacrificing his time in order to support me throughout this project. Finally, I would like to thank Dr. Susan McRae for her constant support through this process.

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Introduction

Advances in medicine and technology are allowing the population to continue to reach a higher age. According to the World Health Organization, the number of people aged 60 years and older is projected to increase from 605 million to 2 billion, between 2000 and 2050 (World Health Organization, 2012). In the United States it is projected that by 2050 one in five Americans will be part of this elderly population (DHHS-GOV, 2005). For example, in the US it is projected that 4.2 million people aged 85 years and older in 2000 will increase five-fold by 2040 (DHHS-GOV, 2005).

While the mechanisms for aging are not fully understood, the overall consensus is that aging is a complex composite of several different processes that can be distinguished from age-related diseases (e.g. Alzheimer's, Parkinson's) (Helfand & Inouye, 2002). The gradual homeostatic failure at the cellular and the organismic level (Masoro, 1995) involves the post-developmental deterioration of physiological, cognitive and emotional performances, and is additionally characterized by the declining ability of a system or an individual to respond to changes and to stress (Masoro, 1995; Whalley, 2001). This reduction of the body's capabilities to respond to changes leads then to an increasing homeostatic imbalance and an increased risk of disease. Moreover, while age-related diseases and, in particular, dysfunctions of the nervous system generally occur with an onset only late in life (DeKosky & Marek, 2003), aging is a continuous process that starts in humans at a relatively early age (between 30 and 40 yrs) and continues until end of life with a nearly constant linear decline of physiological functions (Masoro, 1995).

One of the most notable dysfunctions developing with normal aging is a gradual decline in motor function. This change in motor behavior in the elderly is shown as reduced speed and motor activity, slowed reaction time, impaired coordination (Ropper & Samuels, 2009), slowed initiation of movement (Newman, LeWitt, Jaffe, Clane, & Larsen, 1985; Morgan, Phillips, Bradshaw, Iansek, & Bradshaw, 1994), slowed execution of movement (Morgan, Phillips, Bradshaw, Iansek, & Bradshaw, 1994), shuffling gait (McGeer, McGeer, & Suzuki, 1977; Newman, LeWitt, Jaffe, Clane, & Larsen, 1985), deterioration of gait (Ropper & Samuels, 2009), and other motor signs involving a general poverty of movement (Mortimer, 1988; Critchley, 1956). In particular, as age increases a decreased stability may result in a slower walking pace with shorter steps in order to maintain balance while walking (Ropper & Samuels, 2009; Mortimer, 1988). Consequently, a daily task such as walking becomes much more difficult and may contribute to the loss of independence by the elderly (Mortimer, 1988; Joseph & Roth, 1988).

The striatum, consisting of the caudate nucleus and putamen in the basal ganglia of the brain, is a key structure in the central nervous system involved in motor control (Kandel, Schwartz, &Jessell, 1995) (Figure 1). The striatum is composed to ~90% of medium spiny neurons (MSNs). The striatum is has two types of MSNs. MSN projecting from the striatum to the Globus Pallidus (striato-pallidal) MSNs tend to express D2 receptors, while D1 receptors are highly expressed on striato-nigral MSNs (Surmeier, et al., 2010). The striatum is innervated by the substantia nigra (SN), the main dopamine (DA) producing-structure in the brain, and these innervations form the nigrostriatal dopaminergic system

The Striatum

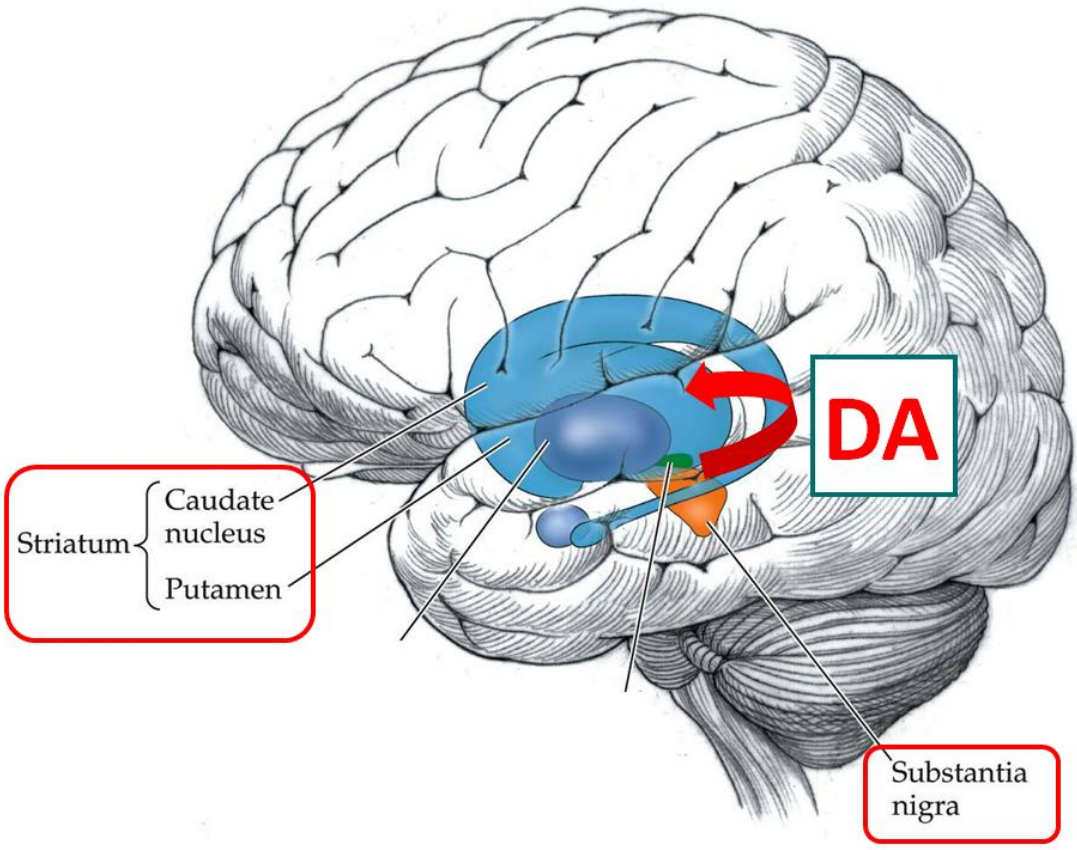

Figure 1. Motor function is controlled largely by the brain. Within the brain the striatum, shown in blue, is the main relay for motor coordination, and it is composed of the Caudate Nucleus and the Putamen. The striatum is heavily innervated by the Substantia nigra (SN), shown in orange. Neurons originating from the SN are dopamine producing or dopaminergic neurons. These neurons form the nigrostriatal pathway.

(Bové, Prou, Perier, &Przedborski, 2005). The basal ganglia and the striatum execute intentional motor programs and suppress other unintentional motor programs (Kandel, Schwartz, &Jessell, 1995; Darbin, 2012). The basal ganglia are also thought to play a role in executing complex motor programs or movements, along with "planning, initiation, and control of voluntary movement (Darbin, 2012)" (Joseph & Roth, 1988).

The importance of the striatum in motor function is evident, as pharmacological or surgical lesions of the striatal dopaminergic system in animals mimicked Parkinson's disease (PD) (Joseph & Roth, 1988). PD has been characterized as the loss of nigral dopaminergic cells in the nigrostriatal pathway, which leads to a depletion of DA (Darbin, 2012; Bové, Prou, Perier, & Przedborski, 2005; Duty & Jenner, 2011; Joseph & Roth, 1988; Mortimer, 1988). The compounds reserpine and alpha-methyl-p-tyrosine are used to mimic the neurochemistry of PD by significantly decreasing the DA content of the SN (~85%) and the striatum (>95%) (Duty & Jenner, 2011). This decrease in DA content causes changes in motor behavior such as akinesia (Duty & Jenner, 2011), compromised postural tone (Joseph & Roth, 1988), and hind leg rigidity (Duty & Jenner, 2011). Neurotoxins 6-hydroxydopamine (6-OHDA) and N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) have been used in animals to induce Parkinsonism. 6-OHDA injected into nigrostriatal fibers degenerates dopaminergic neurons, which can cause akinesia, limb dysfunction, rigidity, and impaired orientation to sensory stimuli (Joseph & Roth, 1988; Duty & Jenner, 2011). MPTP, intravenously injected, also degenerates dopaminergic nigrostriatal neurons, causing tremor, rigidity, slowness of movement, postural instability, and freezing (Bové, Prou, Perier, & Przedborski, 2005). In addition to the clinical evidence of PD showing that the striatal DA

system is involved in changes in motor function with age, studies have shown that normal aging leads to neurochemical changes in the striatum. According to a study by McGeer et al. (1977), the enzyme tyrosine hydroxylase (TH) shows a significant decrease with age in the striatum, in addition to an aging-related loss of nigral dopaminergic cells innervating the striatum. TH is an enzyme involved in the synthesis of DA, and can serve as a neurochemical biomarker for DA synthesis. They showed that the 400,000 nigral cells at birth decline to less than 200,000 by age 75. Other studies have extrapolated that by the age 100 dopaminergic nigral cell count would be 140,000, which is comparable to the nigral cell counts of PD patients at 60,000-120,000 (Joseph & Roth, 1988). Thus it is evident that the striatal dopaminergic system is an important part of motor control and aging.

DA is a catecholaminergic neurotransmitter derived from the amino acid tyrosine (Beaulieu &Gainetdinov, 2011). DA and DA producing neurons are mainly located in the brain (Missale, Nash, Robinson, Jaber, & Caron, 1998; Beaulieu &Gainetdinov, 2011). Within the brain 4 major DA pathways have been identified, the nigrostriatal, mesolimbic, mesocortical, and the tuberoinfundibular pathways (Beaulieu &Gainetdinov, 2011). DA is involved in a variety of functions within the Central Nervous System (CNS). For example DA plays a role in motor function, voluntary movement, cognition, emotion, positive reinforcement, food intake, and endocrine regulation (Beaulieu &Gainetdinov, 2011).

DA actions are mediated by five G protein-coupled DA receptors, D1 through D5 (Beaulieu &Gainetdinov, 2011). This family of DA receptors is divided into two classes, the D1-like and D2-like receptors (Figure 2 A). The D1-like receptors consist of DA receptors

D1 and D5. The D2-like receptors consist of DA receptors D2, D3, and D4. The D1-like and D2-like division has somewhat disparate molecular structures. The D1-like receptors D1 and D5 are 80% homologous in receptor structure, while the D2-like receptors D3 and D4 are 75% and 53% homologous to the D2 receptor, respectively (Beaulieu &Gainetdinov, 2011) There is strong evidence for both receptor families in the striatum (for example: (Beckstead, 1988 ; Broaddus & Bennett Jr., 1990; Gerfen, et al., 1990; Levey, et al., 1993; Aizman, et al., 2000; Chu, Wilczynski, & Wilcox, 2001; Bertran-Gonzalez, et al., 2008)). Functionally DA receptors differentially regulate second messenger pathways by either increasing (D1-like: through the coupled $G_{s/olf}$ G protein) or decreasing (D2-like: through the coupled $G_{i/o}$ G protein) the activity of adenylate cyclase (AC) (Beaulieu &Gainetdinov, 2011). Additionally, the affinity of the different receptor subtypes towards DA is D3>D4>D2~D5>>D1 (data compiled from http://pdsp.med.unc.edu/). Thus based on the DA receptor subtypes affinity for DA, at low levels of DA release, inhibitory actions promoted by D2-like receptors are likely to dominate overall DA effects while excitatory effects mediated by D1-like receptors are likely to occur only at higher DA doses (Barrière, Mellen, &Cazalets, 2004; Han& Whelan, 2009) (Figure 2 B and C). A recent study demonstrated such dose-dependent effects of the different DA receptor subtypes on the motor systems when tested in the isolated spinal cord (Clemens, Belin-Rauscent, Simmers, & Combes, 2012).

Dopamine Receptor Affinities

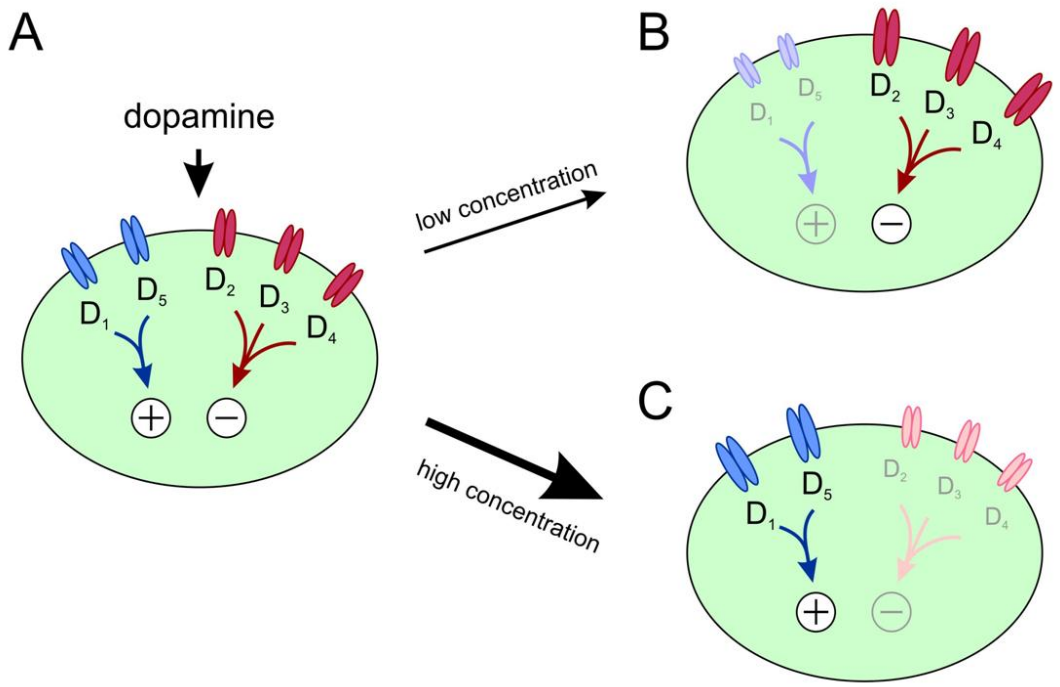

Figure 2. **(A)** Dopamine is a neurotransmitter and its actions are mediated by DA receptors D1 through D5. This family of DA receptors is divided into two classes the D1-like (blue) and D2-like receptors (red). D1-like receptors consist of D1 and D5 receptors and they tend to mediate excitatory actions. While D2-like receptors consist of D2, D3, and D4 receptors and they tend to mediate inhibitory actions. Based on previous studies it has been shown that D2-like receptors have a higher affinity for DA when compared to D1-like receptors. **(B)** Therefore at low concentrations of D2-like receptors mediate inhibitory actions. **(C)** In contrast, at higher concentrations of DA, D1-like receptors are additionally activated and their excitatory actions can overcome the inhibitory effects mediated by the D2-like receptors.

With age, there is a decline in expression of the striatal DA system that is independent of diseases such as PD (Reeves, Bench, & Howard, 2002; Collier, et al., 2007; Ishida, Okawa, Ito, Shirokawa, & Isobe, 2007; Jucaite, Forssberg, Karlsson, Halldin, &Farde, 2010), but there are no data available that address if these changes in the DA system are also mirrored at the receptor level in the striatum. As D1-like and D2-like receptors mediate opposite effects, and as striatal D1, D2, and D3 receptors are the primary receptors involved in motor function (Beaulieu & Gainetdinov, 2011; Missale, Nash, Robinson, Jaber, & Caron, 1998), we hypothesized that aging-related alterations in the balance of expression levels for D1-like and D2-like receptors might be associated with some of behavioral changes observed in the elderly.

Therefore, in order to test this hypothesis we determined here the protein receptor expression levels of select DA (D1, D2, and D3) receptors in the striatum of differently aged mice.

Materials &Methods

Animals

All experimental procedures were approved by the East Carolina University Institutional Animal Care and Use Committee (IACUC, AUP Q273a). Male C57BL/6 mice aged at 2 months (n=4), 1 year (n=4) and 2 years (n=4) were used. Animals were deeply anesthetized and decapitated, and the brains were removed from the skull and stored in a tube containing RNAlater (Ambion AM7021). The samples were then stored in a -20° C freezer.

Striatal Dissection

Striatal tissue was dissected out according to the Richfield1 protocol (The Jackson Laboratory, 2003). In this study only the left hemisphere was used to harvest the striatal tissue, the other hemisphere was stored for additional later analyses in RNAlater at -20° C. Briefly, the brain was oriented in a dorso-ventral orientation and the cerebellum was dissected off. Then, the midline of the brain was cut to separate the brain into right and left hemispheres. The cortex was then carefully peeled off, followed by removing the hippocampus. Finally, the striatal tissue obtained was stored in Eppendorf tubes containing RNAlater at -20° C for later analysis.

Protein Preparation

After obtaining the striatal tissue, the protein concentrations were determined for each sample. First, the cell lysis solution was prepared with RIPA buffer, protease inhibitor, and phosphatase inhibitor. Then, the striatal samples were placed in test tubes, on ice, containing

the cell lysis solution. Next, the samples were sonicated and centrifuged for 20 minutes at >16,000 x g. The supernatant containing the protein was transferred to new Eppendorf tubes and stored at -80° C. Striatal protein concentrations were determined using the EZQ Protein Quantification Kit for gel electrophoresis (Molecular Probes, Life Technologies, Grand Island, NY).

Gel Electrophoresis

After determining striatal protein concentrations we performed western blotting of the samples. These experiments were done in replicates with different sample layouts and order of antibody exposure per gel, to ensure reproducibility and verify consistencies. First, a master mix of Laemmli Sample Buffer (Bio-Rad, Hercules, CA) was made, by adding β-mercaptoethanol to Laemmli Sample Buffer at a 1:20 dilution. Next, each sample was prepared with 30 µg of protein, 20 µl of the sample buffer, and RIPA buffer to bring up the volume to 40 µl. Then, the samples were boiled for 10 minutes at 95-100° C and loaded into an 18-well Criterion TGX Any kD precast gel (Bio-Rad, Hercules, CA). After the gels were loaded and the running buffer (25 mM Tris, 192 mM glycine and 0.1% Sodium Dodecyl Sulfate) was poured into the gel running apparatus, the protein samples were ran at 100 V until the tracking dye reached the bottom of the gel, usually after 2 hours.

Transfer

Upon completion of the protein samples migrating down the gel, the transfer of protein from the gel to an Immobilon-P PVDF transfer membrane (Millipore, Billerica, MA) was performed. First, in each transfer, the membranes were activated by a five-minute incubation

in methanol. This procedure was followed by preparing the transfer sandwich containing the fiber pads, filter paper, gel, and membrane. The sandwich was then placed into the transfer apparatus containing chilled transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol). Finally, the electrophoretic transfer ran for one hour at 100 V.

Antibody Probing

After the transfer, the membranes were probed for DA receptors D1, D2, D3, and ß-actin as a loading control. First, membranes were placed in a non-fat 5% milk in Tris buffered saline plus Tween blocking solution (150 mM NaCl, 50 mM Tris, 0.1% Tween-20, pH 7.4) at 4° C overnight. Primary antibody was then prepared in blocking solution and incubated overnight at 4° C. The membranes were then washed with Tris buffered saline plus Tween (TBS-T) three times before being incubated in secondary antibody. Secondary antibody was also prepared in fresh blocking solution. After a one-hour incubation the membranes were washed three times again with TBS-T. Then, at room temperature, Amersham ECL Plus Western Blotting Detecting Agents (Amersham, GE Healthcare, Pittsburgh, PA) was applied to the membrane and incubated in the dark for five minutes, followed by exposing membranes to Amersham Hyperfilm Blue (Amersham, GE Healthcare, Pittsburgh, PA). After successfully probing for a given protein, the membranes were stripped with stripping buffer containing 60 mM Tris, 2% Sodium Dodecyl Sulfate, and 0.7% β-mercaptoethanol with a pH 6.7 at 50° C. The membranes were then washed in TBS-T five times or until the stripping buffer has been completely dissipated. The process was then repeated until all proteins were successfully probed.

Antibodies & Detection

For this study, the following primary antibodies to probe for DA receptor expression and ß-actin were used:

D1: Dopamine D1 Receptor (ab78021, abcam, Cambridge, MA, used at 1:500)

D2: Dopamine D2 Receptor (ab21218, abcam, Cambridge, MA, used at 1:800)

D3: Dopamine D3 Receptor (ab42114, abcam, Cambridge, MA, used at 1:2500)

ß-actin: ß-actin Loading Control (ab8227, abcam, Cambridge, MA, used at 1:2000)

Secondary HRP-tagged secondary antibodies (goat anti mouse (HAF007)) or goat anti rabbit (HAF008, both R&D Systems, Minneapolis, MN) was then used at a dilution of 1:2000 to detect the primary antibody binding.

Analysis

After exposure to the secondary antibody, membranes were photographed and scanned, and images were analyzed with a software package Image J (provided by the National Institutes of Health), and subsequent statistical analyses were performed with SigmaPlot (version 11). Differences between striatal DA receptors D1, D2, and D3 protein expression levels in the 2 month, 1 year, and 2-year-old animals were determined using one-way ANOVA, followed by appropriate post-hoc comparisons.

Results

ß-*Actin*:

ß-actin, the loading control, allowed us to normalize levels of protein detected by confirming that protein loading was the same across the gel. An immunoreactive band at an approximate molecular weight of 37 kD was analyzed to yield protein expression levels of ß-actin (Figure 3 A). Protein expression of ß-Actin expression remained stable and did not significantly change with increasing age (Figure 3 B, $p = 0.669$).

Effects of Aging on Striatal D1 Receptor protein expression levels:

An immunoreactive band at an approximate molecular weight of 50 kD was analyzed to yield protein expression levels of the striatal D1 receptor (Figure 4 A). Striatal D1 receptor protein expression levels significantly changed with increasing age. Protein expression levels increased significantly by approximately 4-fold from the 2 month old animals (101.0 ± 12.9 %) to the 1 year old animals (383.2 ±62.4 %), and approximately 5-fold from the 2 month old animals to the 2 year old animals (474±49.5 %, $p = <0.001$). Overall, there was a significantly increased expression of the D1 receptor in the striatum with increasing age (Figure 4 B).

ß-Actin
Protein Expression Levels

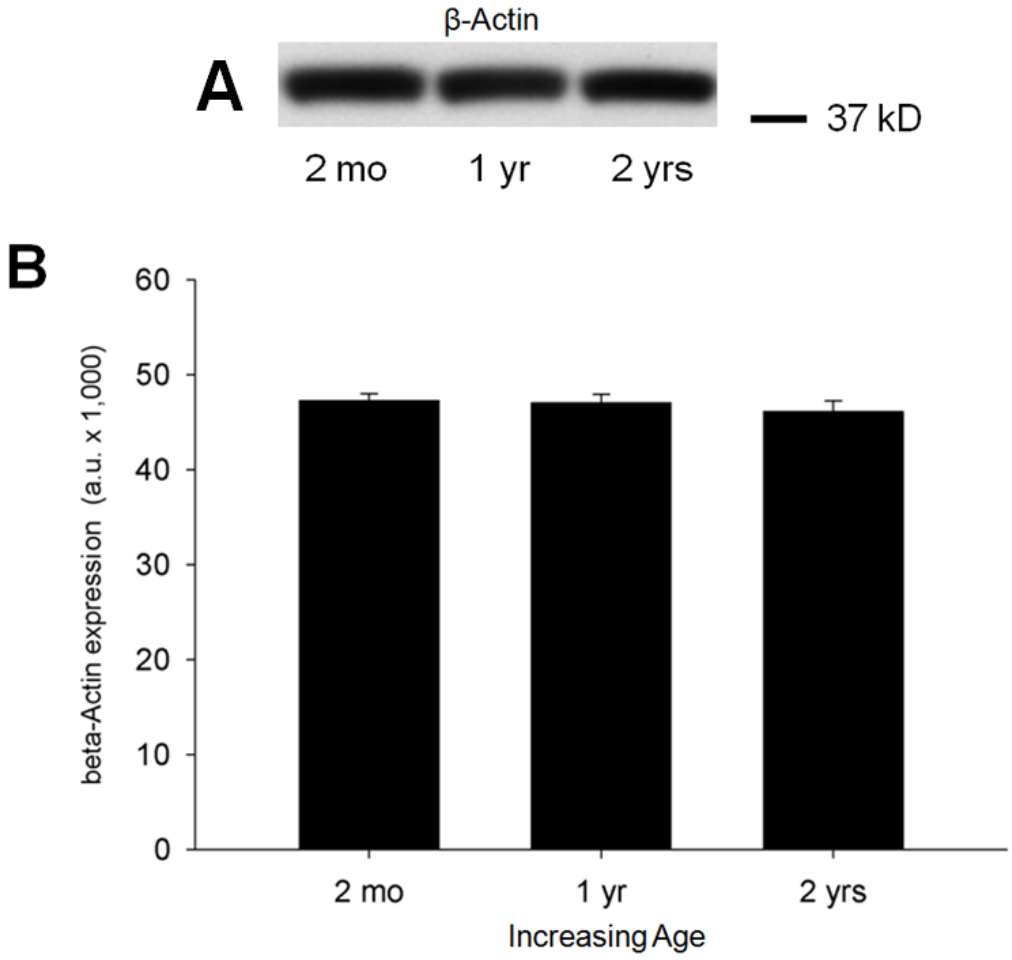

Figure 3. **(A)** Representative x-ray film exposures of 2 month, 1 year, and 2 year old ß-Actin protein bands in the mice striatum. **(B)** Protein expression levels of ß-Actin in the Striatum of animals aged at 2 months, 1 year, and 2 years. A one-way ANOVA test was performed to test for statistical differences. No significant changes were observed with age ($p = 0.669$). Values are represented as mean value ± SE.

Dopamine D1 Receptor
Protein expression levels
Normalized to ß-Actin

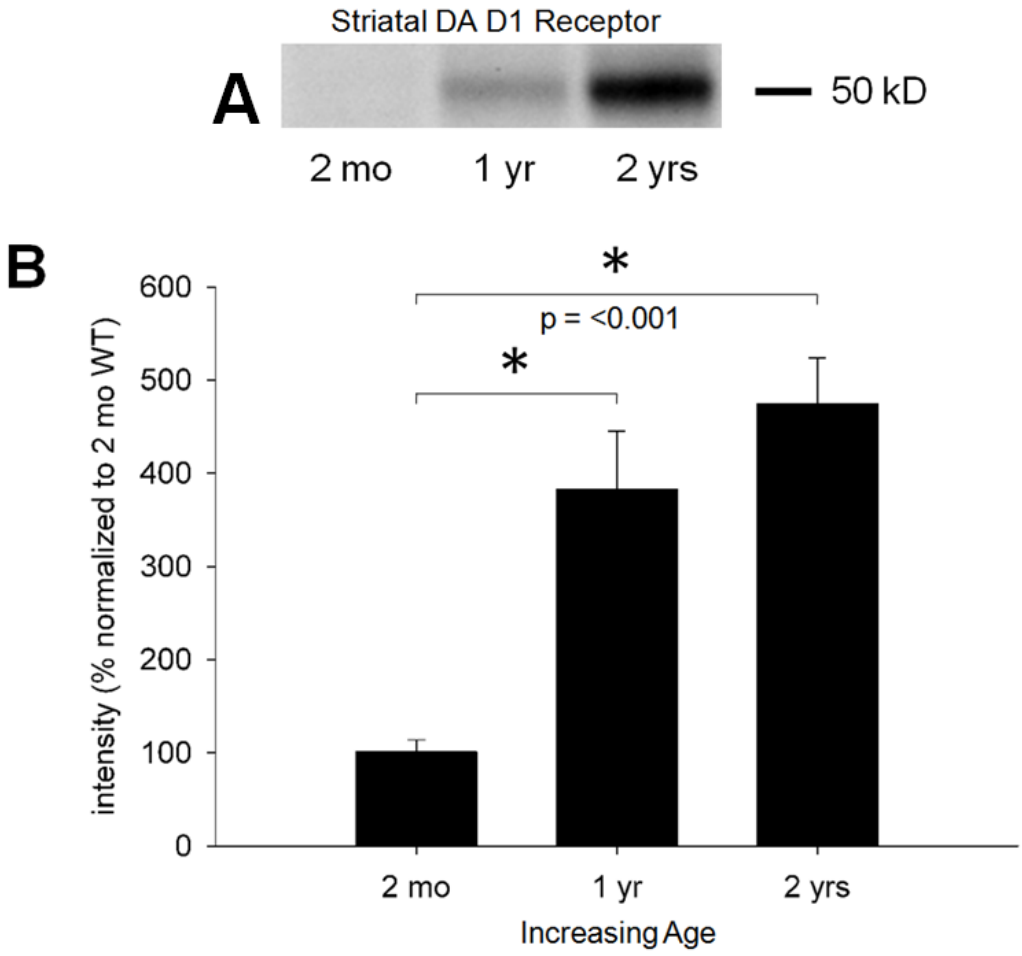

Figure 4. **(A)** Representative x-ray film exposures of 2 month, 1 year, and 2-year-old D1 receptor protein bands in the mice striatum. **(B)** Protein expression levels of D1 receptor expression of animals aged at 2 months, 1 year, and 2 years, normalized to ß-Actin and the 2 month-old animals. A one-way ANOVA and Fischer LSD *post hoc* test was performed to test for statistical differences. Statistical significances are shown with lines and (*) from the 2 month old animals ($p = <0.001$). Values are represented as mean value ± SE.

Effects of Aging on Striatal DA D2 Receptor protein expression levels:

Next, striatal D2 receptor protein expression levels were obtained from the analysis of an immunoreactive band at an approximate molecular weight of 50kD (Figure 5 A). A subsequent quantification revealed that, while we observed a slight increase in D2 receptor expression with age, this increase was not significant (2 month: 100.0 ± 15.2 %; 1 year: 110.8 ±2.81 %; 2 year: 121.0 ± 17.0 %, $p = 0.556$, Figure 5 B).

Effects of Aging on Striatal DA D3 Receptor protein expression levels:

Finally, striatal D3 receptor protein expression levels were obtained from the analysis of an immunoreactive band at an approximate molecular weight of 37kD (Figure 6 A). As for the D2 receptor expression, we did not detect any overall significant difference detected with increasing age form the 2 month old animals, despite a transient strong increase from 2 months to 1 year (2 month: 99.6± 17.8 %;1 year: 147.1± 6.83 %; 2 year: 122.1 ± 11.4 %, $p = 0.078$, Figure 6 B).

Effects of Aging on Striatal DA Receptor Ratio:

Based on the expression data reported in Figures 4-6, we next determined the receptor expression ratio between D1/D2 and D1/D3. We found that both ratios showed an approximate 4-fold increase with increasing age (Figure 7).

Dopamine D2 Receptor
Protein expression levels

Normalized to ß-Actin

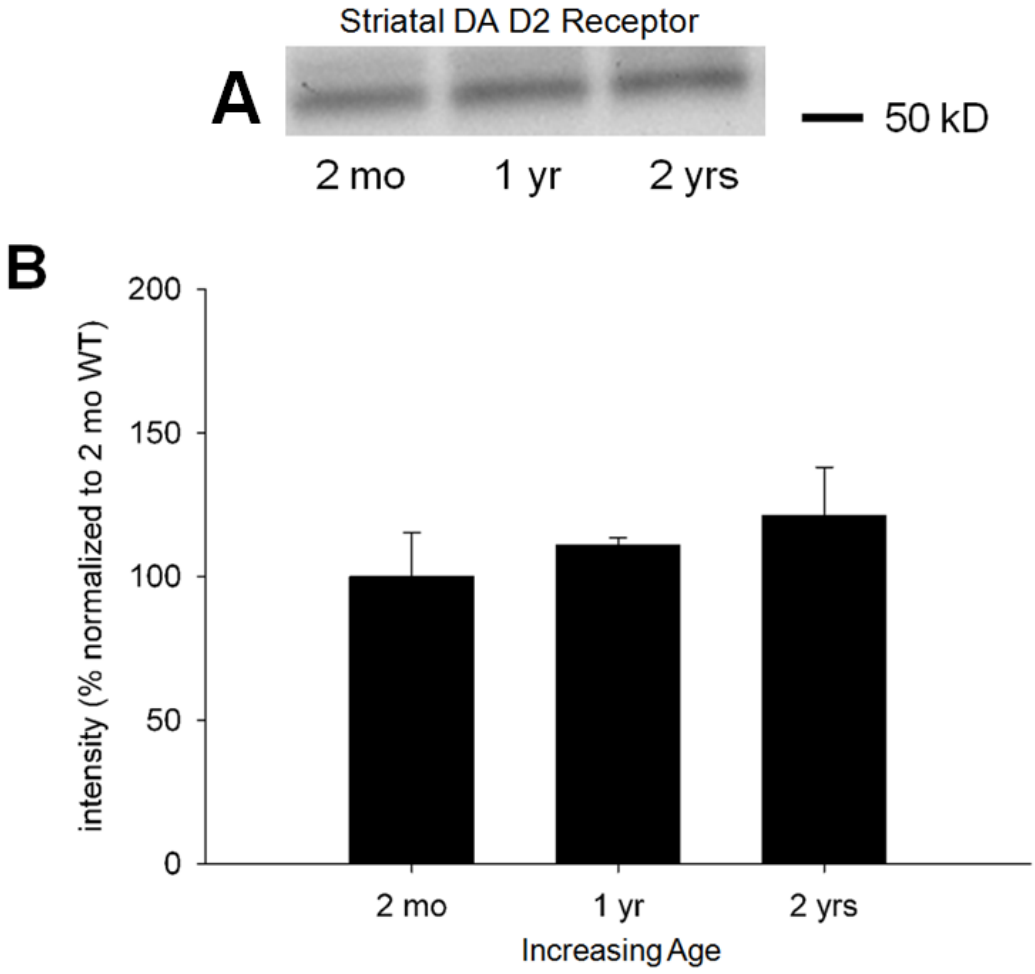

Figure 5. **(A)** Representative x-ray film exposures of 2 month, 1 year, and 2-year-old Striatal D2 receptor protein bands in the mice striatum. **(B)** Protein expression levels of striatal D2 receptor expression of animals aged at 2 months, 1 year, and 2 years, normalized to ß-Actin and the 2 month-old animals. A one-way ANOVA was performed to test for statistical differences. No statistical significance was observed ($p = 0.244$). Values are represented as mean value ± SE.

Dopamine D3 Receptor
Protein expression levels
Normalized to ß-Actin

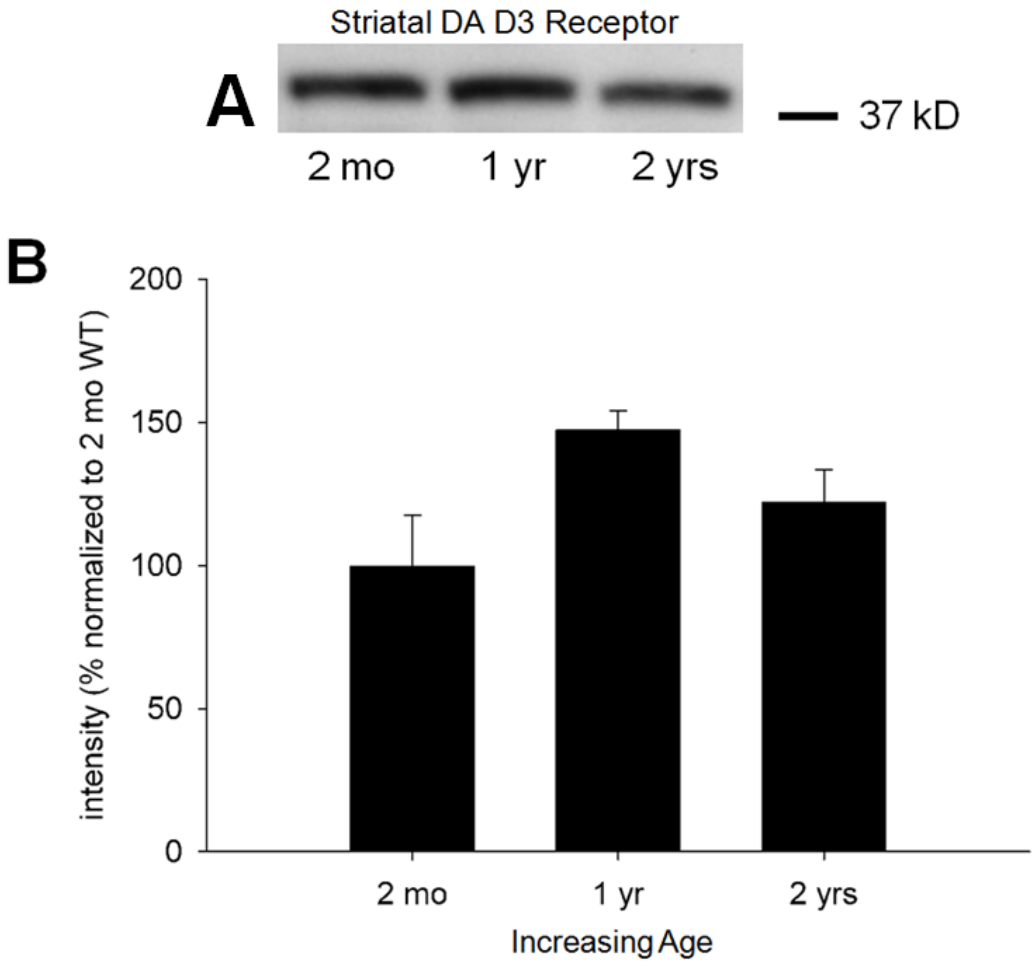

Figure 6. **(A)** Representative x-ray film exposures of 2 month, 1 year, and 2 year old Striatal D3 receptor protein bands in the mice striatum. **(B)** Protein expression levels of striatal D3 receptor expression of animals aged at 2 months, 1 year, and 2 years, normalized to ß-Actin and the 2 month-old animals. A one-way ANOVA was performed to test for statistical differences. No statistical significance was observed ($p = 0.078$). Values are represented as mean value ± SE.

Dopamine Receptor Ratio

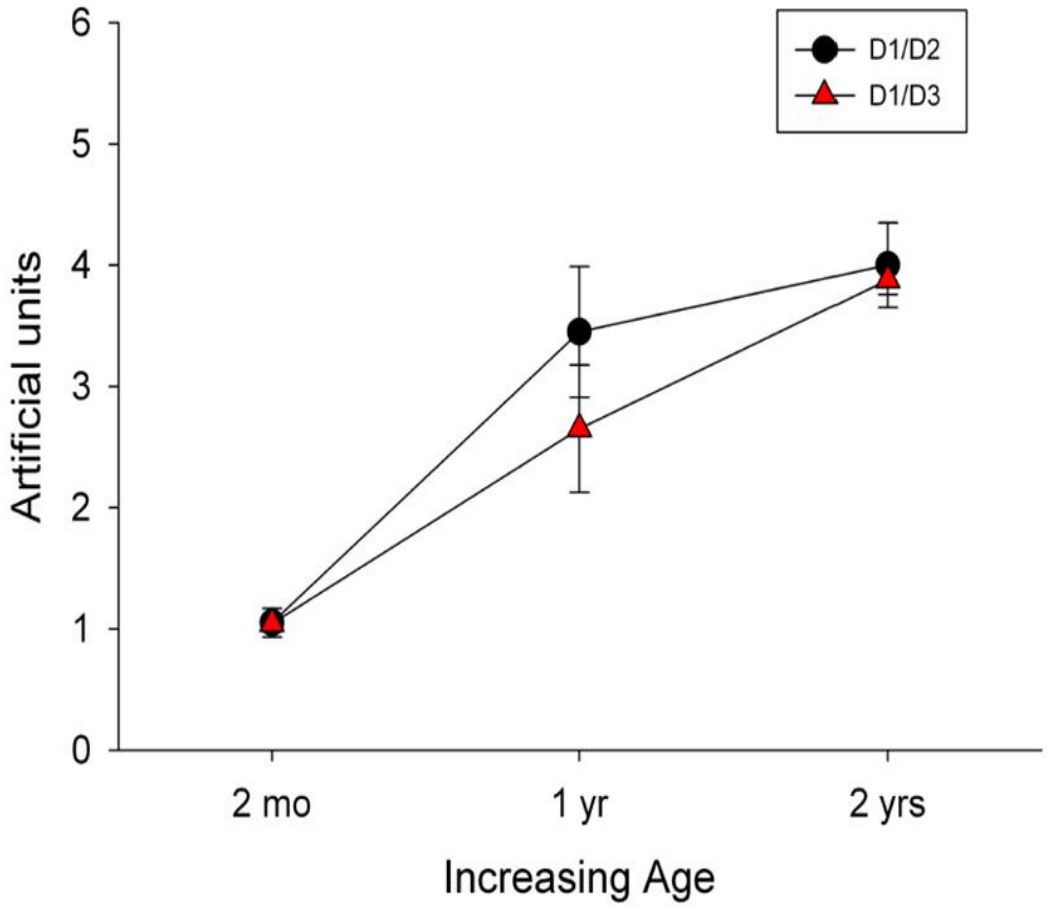

Figure 7. Ratios of D1/D2 and D1/D3 receptor expression levels of 2 months, 1 year, and 2 year old mice striatum. BothD1/D2 and D1/D3 ratios showed an approximate 4-fold increase from the 2-month-old animals to the 2 year old animals.

Discussion

The aim of this study was to examine aging-related changes in protein expression levels of striatal D1, D2 and D3 receptors. In this study we used an aging mouse model to dissect out striatal tissue from animals aged at 2 month, 1 year, and 2 years and to determine through western blotting techniques the corresponding DA receptor expression levels.

The major findings of this study showed a significant increase in striatal D1 receptor protein expression levels with age, while we did not observe any significant changes in either D2 or D3 receptor protein expression levels with age. Thus these findings show an aging-related increase in the balance or ratio of protein expression levels for the D1-like and D2-like receptors in the striatum. As DA-mediated actions via D1 receptors are predominately excitatory, while those mediated through D2 and D3 receptors are predominately inhibitory, this increase in the ratio of D1-like/D2-like protein receptor expression levels suggests that DA-mediated actions in the striatum may undergo a shift towards overall excitation with age (Figure 7). This shift at the receptor level may play a role in normal aging-related changes in motor-coordination observed in the non-diseased elderly.

Increased striatal D1 receptor expression levels

The significant increase in DA D1 receptor expression levels with age shown in this study adds to various aging-related changes seen in different studies. In a study by Morgan et al. (1987) an increased striatal D1 receptor density with age in humans was reported, along with an age-related decline in DA levels. In addition, preliminary data from previous

experiments done in out lab have also confirmed an age-related increase in spinal cord D1 receptor protein expression levels. In contrast, studies in humans have reported an age-dependent decline in striatal D1 receptor (Jucaite, Forssberg, Karlsson, Halldin, & Farde, 2010; Wang, et al., 1998). In rats a ~30% decline in striatal DA D1 receptor densities has been observed with age (Henry, Filburn, Joseph, & Roth, 1986). Henry, Filburn, Joseph, and Roth (1986) also examined a small sample of mice which showed no significant age-related change in striatal DA D1 receptor. Many others have also reported aging-related declines in rat striatal D1 receptor and D1-like receptors binding potentials (Suzuki, Hatano, Sakiyama, Kawasumi, Kato, & Ito, 2001) and mRNA (Zhang & Roth, 1997). Araki, Kato, Shuto, Fujiwara, and Itoyama (1997) also reported that in rats no significant change was observed in binding sites for the striatal D1 receptor with age. It is important to note that the studies reporting a decrease in D1 receptor expression based their findings on different experimental and functional paradigms, while our study shows that when using Western blot approaches to assess striatal D1 receptor protein expression levels, there is a significant age-dependent increase. Alternatively, it is also possible that our small sample size of n=4 for each condition may have biased our findings.

No significant change in D2 and D3 Receptor expression levels

In the present study, no significant age-related changes were observed in striatal DA D2 and D3 receptors. While examining mRNA levels of rat striatal D2 receptor a study, similarly, reported no significant change with age. However, they did report a decreased number of binding sites for the D2 receptor with age (Sakata, Farooqui, & Prasad, 1992). Another study examining binding sites of rat striatal D2 receptor with age showed no

significant change (Araki, Kato, Shuto, Fujiwara, & Itoyama, 1997). In the rat striatum many other studies have also shown decreased levels of striatal D2 receptor mRNA (Mesco, Carlson, Joseph, & Roth, 1993; Valerio, Belloni, Gorno, Tinti, Memo, & Spano, 1994; Mesco, Joseph, Blake, & Roth, 1991; Zhang, Ravipati, Joseph, & Roth, 1995) and binding sites (Henry, Filburn, Joseph, & Roth, 1986; Morgan, et al., 1987; Antonini & Leenders, 1993; Volkow, et al., 1998; Suzuki, Hatano, Sakiyama, Kawasumi, Kato, & Ito, 2001) with age. Henry, Filburn, Joseph, and Roth (1986) also examined a small sample of mice, which showed a significant decrease in binding sites for the striatal D2 receptor with age. While different studies have had varying results, this study is directly examining protein expression levels of the striatal D2 receptor using a Western Blotting technique, which showed no significant change with age. However, due to our small sample size possible significant trends may have not been detected.

Similar to our study, in rats it has also been shown that striatal DA D3 receptor mRNA levels does not change with age (Valerio, Belloni, Gorno, Tinti, Memo, & Spano, 1994).Suzuki, Hatano, Sakiyama, Kawasumi, Kato, and Ito (2001) have also shown that D2-like receptors binding sites decrease with age in the rat striatum. Overall, similar to the D1 receptor the striatal DA D2 appears to vary in terms of expression in different experimental models and through different techniques. Relatively few stuides have been done on the striatal DA D3 receptor protein expression levels with age, so our study provides important data with the Western Blotting technique.

Theory

In our hypothesis we think that the changes in protein expression levels of D1, D2, and D3 seen in this study may be due to the affinity and changes in DA levels with age. An aging-related decline in striatal DA levels has been previously reported in many studies (Umegaki, Roth, & Ingram, 2008; Yue, Zeng, Wu, Yi, Zhang, & Chan, 2012; Wang, et al., 1998; Carlsson & Winblad, 1976; Adolfsson, Gottfires, Roos, & Winblad, 1979; Severson, Marcusson, Winblad, & Finch, 1982; Haycock, Becker, Ang, Furukawa, Hornykiewicz, & Kish, 2003). From these previous findings and the results of this study, we hypothesize the increased striatal DA D1 receptor protein expression might be a response to the decreased levels of DA in the striatum with age and the lower affinity for DA when compared to the D2-like receptors (Figure 2). In contrast, due to the higher affinity of the D2-like receptors (D3 and D2), the decreased DA levels with age might still be sufficient high to activate them. Therefore, the changes seen in D1-like and the lack of changes in D2-like receptors may be caused by body's attempt to maintain DA signaling homeostasis with age.

For future studies, a pharmacological approach may be a possible route worth exploring. For example, in this study we have reported an aging-related increase in the protein expression levels of the striatal DA D1 receptor in mice. With this knowledge the question arises if a D1 agonist was administered to the elderly mice, would the result be similar in young mice? We hypothesize that the increased expression of the D1 receptor with age might lead to a differential treatment effect in young vs. old mice that might be detected behaviorally. Thus it may be necessary to adjust the dosages for DA treatment in an age-appropriated manner.

In summary we saw an aging-related increased expression of the D1 receptor leading to a possible shift in the balance D1-like/D2-like receptor ratio towards an overall excitation in the striatum, which may be associated with changes in motor function observed in the elderly. Further research is needed in the aging mouse model to confirm these results and elaborate on the mechanism of how this shift in the balance of D1-like/D2-like receptors may be associated with the observed changes in motor function. The mechanism behind the increase in D1 receptor expression must also be further explored. This research may eventually lead to a possible therapy in order to maintain DA receptor levels with age or change DA receptor expression in the elderly.

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