HHS Public Access

Author manuscript

Exp Eye Res. Author manuscript; available in PMC 2022 October 01.

Published in final edited form as:

Exp Eye Res. 2021 October ; 211: 108732. doi:10.1016/j.exer.2021.108732.

Effects of D-Serine Treatment on Outer Retinal Function

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Abstract

The role of the N-Methyl-D-Aspartate Receptor (NMDAR) in the outer retina is unclear despite expression of the NMDAR-complex and its subunits in the outer retina. The flash-electroretinogram (fERG) offers a non-invasive measurement of the retinal field potentials of the outer retina that can serve to clarify NMDAR contribution to early retinal processing. The role of the NMDAR in retinal function was assessed using a genetic mouse model for NMDAR hypofunction ($SR^{-/-}$), where the absence of the enzyme serine racemase (SR) results in an 85% reduction of retinal D-serine. NMDAR hypo- and hyperfunction in the retina results in alterations in the components of the fERG. The fERG was examined after application of exogenous D-serine to the eye in order to determine whether pre- and post-topical delivery of D-serine would alter the fERG in $SR^{-/-}$ mice and their littermate WT controls. Amplitude and implicit time of the low-frequency components, the a- and b-wave, were conducted. Reduced NMDAR function resulted in a statistically significantly delayed a-wave and reduced b-wave in $SR^{-/-}$ animals. The effect of NMDAR deprivation was more prominent in male $SR^{-/-}$ mice. A hyperfunction of the NMDAR, through exogenous topical delivery of 5mM D-serine, in WT mice caused a significantly delayed a-wave implicit time and reduced b-wave amplitude. These changes were not observed in female WT mice. There were temporal delays in the a-wave and amplitude and a decrease in the b-wave amplitude and implicit time in both hypo- and NMDAR hyperfunctional male mice. These results suggest that NMDAR and D-serine are involved in the retinal field potentials of the outer retina that interact based on the animal's sex. This implicates the involvement of gonadal hormones and D-serine in retinal functional integrity.

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This study is part of the PhD thesis of Nathalia Torres Jimenez.

All authors: No conflict of interest

Keywords

electroretinogram; D-serine; mice; a-wave; b-wave; mesopic

Introduction

The N-Methyl-D-Aspartate receptor (NMDAR) is an ionotropic glutamate receptor essential for synaptic plasticity and learning and memory in the brain (Malenka and Bear, 2004). One of the unique characteristics of this receptor is that it requires a second ligand other than glutamate to activate the channel (Kleckner and Dingledine, 1988; Paoletti, 2011); this makes glycine and D-serine, the two co-agonists, indispensable for NMDAR functioning. In the retina, NMDARs are known to contribute to the light-evoked response of retinal ganglion cells (RGCs) (Cohen and Miller, 1994; Massey and Miller, 1990), mediate GABAergic feedback (Ferreira et al., 1994), and excitotoxicity (Vorwerk et al., 2000). Unfortunately, most of the knowledge about NMDAR functionality in the retina is focused on the retinal ganglion cell (RGC) layer. However, functional NMDARs have been found in horizontal cells in catfish (O'Dell and Christensen, 1989), but were non-functional in mammals (Kalloniatis et al., 2004). In addition, NMDAR subunits have been localized in photoreceptors and cells in the inner nuclear layer (Brandstätter et al., 1994), although these studies did not look for functional receptors. Therefore, it is unclear if the NMDAR plays a role in early retinal processing.

The role of D-serine as an NMDAR co-agonist has increased our understanding of NMDAR function in the retina (Hashimoto et al., 1992a; Hashimoto et al., 1993; Kleckner and Dingledine, 1988; Mothet et al., 2000). D-serine serves as an endogenous co-agonist for the NMDAR in the retina, potentiating NMDAR currents in RGC several fold greater than glycine at the same concentration (Gustafson et al., 2007; Stevens et al., 2003). This increase of NMDAR currents by the addition of D-serine suggested that NMDARs are not saturated, and that release of D-serine would result in recruitment of the NMDAR (Gustafson et al., 2007; Stevens et al., 2003). However, it is as yet unclear how dynamic recruitment of NMDAR through D-serine is initiated by light, and how this recruitment affects upstream retinal function. Studies of D-serine in the retina have been limited to its effects on RGC currents and not on retinal field potentials as a whole. Given that functional NMDARs are located earlier in the vision pathway, D-serine acting on the NMDAR may affect electric potentials in the outer retina. One such electric potential is reflected in the flash-electroretinogram (fERG), a light-evoked potential from the retina in response to a flash of light (Frishman and Wang, 2011). The fERG assesses the integrity of the retina, shedding light on vertical signal processing. The first component of the fERG is known as the a-wave, which represents activity from photoreceptors. The second component is known as the b-wave, which reflects activity from on-bipolar cells (Frishman and Wang, 2011). These components represent retinal field potentials that can be used to investigate the role of NMDAR and D-serine in the outer retina. The electroretinogram offers a quantitative, non-invasive method to examine neural activity under hypo- and hyper-functionality of the NMDAR in the outer retina. We tested the effect of exogenous D-serine treatment on the fERG using mesopic adaptation in control and $SR^{-/-}$ mice to determine its potential

role in neural activity in the outer retina. Our previous study of the ERGs in this system demonstrated visualization of significant changes only in mesopic light adaptation (Torres Jimenez et al., 2020). There is increasing use of mesopic light adaptation protocols to demonstrate changes in both retinal (Feigl and Zele, 2010; Feigl et al., 2011) and other conditions, including hepatic retinopathy (Uhlmann et al., 2003), Duchenne muscular dystrophy (Barboni et al., 2013), and diabetic retinopathy (Tahara et al., 1993). As mesopic illumination is the transition zone between dark- and light-adapted retinas, it provides a mixed rod and cone signal (Krizaj, 2000; Zele and Cao, 2015) that can allow for early identification of retinal conditions that involve both rods and cones.

Methods

Animals

Serine racemase knock out ($SR^{-/-}$) mice were obtained from Dr. Joseph Coyle (Balu and Coyle, 2015), and the colony was maintained by Resource Animal Resources at the University of Minnesota. Littermate controls and $SR^{-/-}$ mice were used at ages 8-16 weeks and included both males and females. A total of 34 mice were used for all experiments, seventeen for each genotype. All procedures were in accordance with the standards provided by the National Institutes of Health for use of animals in research and the animal care and standards set by the Association for Research in Vision and Ophthalmology. All studies were approved by the Institutional Animal Care and Use Committee at the University of Minnesota.

Experimental design and D-serine delivery

In vivo fERGs were conducted in all mice pre- and post-topical delivery of D-serine at a concentration of 5 mM diluted in 0.05% carboxymethylcellulose Refresh Plus Lubricant Eye Drops (Allergan, Inc., Irvine, CA). One day prior to recording, normal Refresh Plus eye drops (0.05%) were topically delivered. On the day of recording, either normal eye drops were administered or eye drops containing 5mM D-serine were used.

Stimulation and recording

Pupils were dilated with tropicamide (1%) eye drops (Akorn, Inc., Lake Forest, IL), followed by proparacaine hydrochloride eye drops (0.5%) (Akorn, Inc.). Mice were anesthetized for the fERG recordings with 4% isoflurane, an anesthetic known to leave the fERG recordings unchanged (Woodward et al., 2007). A contact lens with a coiled wire electrode was placed on the cornea, and methylcellulose eyedrops (1.0%) (Allergan) were used to hold the contact in place and protect the cornea from drying out. The stimulus duration was 4 milliseconds (msec.), and retinas were adapted to a steady white background luminance of 0.35 cd·s/m^2 for 15 minutes prior to recording. The stimulation protocol consisted of four msec. white flashes of 7 incrementing steps ranging from 0.175 to 11.2 cd·s/m^2 were presented on a white background luminance of 0.1 cd/m^2 and adapted for 10 minutes prior to recording. Our previous study demonstrated this protocol to be the most sensitive for demonstrating significant differences (Torres Jimenez et al., 2020). A D215 Espion E^2 Console (Diagnosys LLC, Lowell, MA) was used for data collection under a bandpass filter of 0.3-300 Hz. At each light intensity, four traces were collected, which were

averaged to determine the representative response at each light intensity for each mouse tested (Supplemental Figure 1).

After data collection, a customized MATLAB program was used to remove oscillatory potentials from the a- and b-wave recordings. This was done using a low-pass filter with a passband of 50Hz and a stopband of 65 Hz (Asi and Perlman, 1992; Benchoin et al., 2017). Once filtered, the a-wave amplitude was measured from the pre-flash baseline to the peak of the a-wave, the b-wave amplitude was measured from the peak of the a-wave (trough) to the largest peak of the b-wave, and implicit times of the a-waves and b-waves were measured from flash onset to the peak of the response.

Statistical analysis

A three-way (2x2x2) mixed Analysis of Variance (ANOVA), with two between-subject (Genotype and Sex) and one within-subject factor (Treatment), was conducted at each flash strength to evaluate the interaction between treatment, genotype, and sex. We tested all possible simple two-way interactions that were adjusted with Bonferroni correction at statistical significance set at $p<0.025$. If simple two-way interactions were significant, simple main effects were analyzed. The dependent variables tested were a-wave amplitude and implicit time and b-wave amplitude and implicit time. All analyses were conducted for each light intensity. An IBM SPSS Statistics 26 program was used to conduct all statistical analyses.

Results

Effect of D-serine on the a-wave amplitude

When the a-wave amplitude data from the pre- and post-treatment were analyzed, only the a-wave amplitude from the male WT mice was significantly different from the other cohorts and only at the brightest flash strength. Statistical significance was based on demonstration of a three-way interaction between treatment, genotype, and sex using ANOVA (Table 1). Analysis of simple two-way interactions revealed that at the brightest flash strength, there was an interaction between sex and genotype (Figure 1A, Table 1, Supplemental Figure 1). Surprisingly, this significant difference for the male WT at the brightest flash strength disappeared with the application of exogenous D-serine (Figure 1B, Table 1). Simple main effects analyses examining the four distinct groups did not show statistically significant differences in the a-wave amplitude with exogenous application of D-serine (Table 2). However, the a-wave amplitude of male WT mice showed trends to a reduction in a-wave amplitude with increasing flash strength (Table 2). Also noteworthy is that the a-wave amplitudes of male WT and $SR^{-/-}$ mice became even more similar after D-serine treatment. Female $SR^{-/-}$ mice showed no significant changes at any flash strength. These results suggest that the a-wave amplitude in all groups was unaffected by exogenous addition of D-serine but rather tended to result in the WT and $SR^{-/-}$ responses being more similar to other than pre-treatment.

Effect of D-serine on the a-wave implicit time

In the $SR^{-/-}$ mice, there was a statistically significant delay in the a-wave implicit time compared to WT mice at all but the brightest flash strengths, based on the three-way interaction in the ANOVA (Figure 2A, Table 3). Four of those flash strengths had a statistically significant two-way interaction between treatment and genotype (Figure 2 A, B; Table 3). Pre-D-serine delivery, there was a delay in the a-wave implicit time in the male $SR^{-/-}$ mice compared to the other genotypes (Figure 2C). Post-D-serine application, there was no difference in the implicit time of the a-wave in $SR^{-/-}$ mice in comparison to WT mice (Figure 2D, Table 3). Separating the animals into four distinct groups, as revealed by the simple main effect analyses, demonstrated that the delay in a-wave implicit time observed when both male and female $SR^{-/-}$ a-wave implicit times were combined, prior to topical delivery of D-serine, was driven by the male $SR^{-/-}$ (Figure 3E, Table 3). At the middle flash strengths, only male $SR^{-/-}$ had a statistically significant reduction in the a-wave implicit time when D-serine was added exogenously. These results suggest that adding D-serine eliminated the temporal delay in the a-wave implicit time in male $SR^{-/-}$ that was observed in the pre-D-serine condition (Table 4). In contrast, while not statistically significant, male WT mice trended to have a delay in the a-wave implicit time with exogenous D-serine treatment (Figure 3B). Female WT (Figure 3C) and female $SR^{-/-}$ (Figure 3F) had no temporal changes in the a-wave implicit time when exogenous D-serine was administered (Table 4). These results imply that changes in exogenous D-serine can affect the temporal dynamics of the a-wave in male WT mice, accelerating it in male $SR^{-/-}$ and delaying it, though not significantly, in male WT. In summary, exogenous application of D-serine eliminates the genotype and sex differences observed in the normal pre-treatment retina, making the a-wave implicit time of all four groups essentially the same.

Effect of D-serine on the b-wave amplitude

Unlike the a-wave amplitude, there was a highly significant difference between the b-wave amplitude between WT and $SR^{-/-}$ mice based on an ANOVA for three-way interaction between treatment, sex, and genotype at all flash strengths (Figure 4A, Table 5, Supplemental Figure 1), as well as two-way interactions between treatment and genotype. In addition, at all flash strengths, there was a statistically significant decrease in b-wave amplitude in the untreated male $SR^{-/-}$ mice (Figure 4C, Table 5) based on the analysis of two-way interactions between genotype and sex (Figure 4C, Table 5). Interestingly, with exogenous application of D-serine, the differences in the b-wave amplitude that depended on the animal's genotype and sex disappeared, resulting in a b-wave amplitude that was similar between all cohorts (Figure 4D, Table 5). This result suggests that exogenous D-serine eliminated the difference in the b-wave amplitude that depended on the animal's genotype and sex.

Our simple main effect analysis, which separates the animals into four distinct groups, revealed that exogenous D-serine significantly reduced the b-wave amplitude of male WT compared to the male $SR^{-/-}$ (Figure 5C, D; Table 6). In male WT, exogenous D-serine decreased the b-wave amplitude; this decrease was statistically significant at four dimmest flash strengths (Figure 5C, Table 6). In male $SR^{-/-}$ mice, exogenous D-serine increased the b-wave amplitude; this increase was statistically significant at 3 of the brighter flash

strengths (Figure 5D, Table 6). There were no statistically significant differences in the b-wave amplitude in either female WT or female $SR^{-/-}$ with exogenous application of D-serine (Figure 5 E, F, Table 6). These results demonstrate that the exogenous addition of D-serine affected the b-wave amplitude of only male mice, and the effect was opposite depending on the genotype of the male mice. In male WT, exogenous D-serine reduced the amplitude of the b-wave; while, in male $SR^{-/-}$ exogenous D-serine increased the amplitude of the b-wave. This effect was more prominent at some flash strengths than at others.

Effect of D-serine on the b-wave implicit time

The b-wave implicit time was significantly delayed in the $SR^{-/-}$ mice before treatment based on the ANOVA showing a significant three-way interaction at all flash strengths (Figure 6A, Table 7). At all the flash strengths there was a statistically significant difference between b-wave implicit times before and after D-serine treatment, based on simple two-way interactions between treatment and genotype (Figure 6A, B; Table 7). Exogenous D-serine reduced the temporal delay in the b-wave implicit time for the $SR^{-/-}$ mice (Figure 6B, Table 7). At all flash strengths but one, there were significant differences in the b-wave implicit time based on genotype and sex interaction across all groups both before D-serine treatment (Figure 6C, Table 7) and after D-serine delivery. In fact, the differences between the b-wave implicit time for all groups became relatively the same post D-serine treatment (Figure 6D).

Simple main effect analyses revealed that the b-wave implicit time of the $SR^{-/-}$ decreased after stimulus onset with the addition of exogenous D-serine (Table 8). However, this effect was only statistically significant in two flash strengths in male $SR^{-/-}$ and in one flash strength in female $SR^{-/-}$ (Table 8). B-wave implicit time was not statistically significantly different in any of the WT mice. This result suggests that the significant differences seen in the b-wave implicit times at all light intensities in the grouped data were not dependent on genotype and sex. It appears that D-serine may only affect the temporal dynamics of the $SR^{-/-}$ mice at selected flash strengths (Figure 7).

Discussion

These data demonstrate that D-serine affects the amplitude and timing of the field potentials of the outer retina, reflected in the a- and b-wave. Furthermore, this work shows an interaction between hypo- or hyper-availability of D-serine and sex that has never been reported.

D-serine is the endogenous co-agonist for the glycine site of the NMDAR (Gustafson et al., 2007). D-serine has been demonstrated to potentiate NMDAR currents constantly upon exogenous delivery of D-serine (Stevens et al., 2003). This suggests that the NMDAR co-agonist sites are not saturated, and more D-serine can serve to recruit more NMDAR to light intensity demands (Sullivan and Miller, 2012). This dynamic role of D-serine is possible due to the sodium-dependent glycine transporter type 1 (GlyT1) uptake system that serves to reduce available glycine, preventing competition between the two co-agonists of the NMDAR (Reed et al., 2009). This places D-serine in a critical role for responding to various light demands. Using whole cell recordings in an *in vitro* retinal preparation, the

$SR^{-/-}$ mice showed significantly reduced light-evoked NMDAR currents that were restored by the addition of exogenous D-serine (Sullivan et al., 2011). It is interesting to note that despite the large reduction in the ganglion cell response to light in the $SR^{-/-}$ mice, their optokinetic reflex responses were not significantly different from controls (Sullivan et al., 2011). This may be due to their use of scotopic rather than mesopic adaptation for this test. A study investigating the role of D-serine at different light conditions demonstrated that D-serine enhanced retinal ganglion cell currents at low contrast values, where the stimulus was lower than the low background light (Gustafson et al., 2015). This was further supported by extracellular recording of the proximal negative potential (PNFP), which revealed that at increasing flash strengths, sensitivity of D-serine decreased (Gustafson et al., 2015). This work demonstrated that at low contrast, the co-agonist site of the NMDAR was not saturated, but with increasing light, D-serine occupied the NMDAR co-agonist site in the retinal ganglion cell layer. Given that there are NMDAR subunits in the outer retina - although functional receptors were not specifically studied - (O'Dell and Christensen, 1989; Brandstätter et al., 1994), it is possible that D-serine mediation of the light response occurs earlier in retinal processing. It should be noted that in a study of primate retina, a-wave alterations in photopic conditions appeared to be modulated by post-receptor activity (Bush and Sieving, 1994). Further studies are needed to clarify these effects of D-serine in retinal function. Our current work showed a delay in the temporal property of the a-wave rather than magnitude differences. This delay in the a-wave was a result of excess or deficiency of D-serine that was sex dependent.

Lastly, our work showed a reduction in the b-wave amplitude in both hypo- and hyperfunctional conditions that was also sex dependent. One possible hypothesis for D-serine administration effects on only male mice can be attributed to known effects of testosterone on D-amino acid oxidase (DAAO) (Clark et al., 1943; Konno and Yasumura, 1983). In the kidney, higher DAAO enzyme is found in male mice across all six different mouse strains in comparison to their female counterparts (Konno and Yasumura, 1983). In addition, castration reduced the amount of pyruvic acid, a DAAO by product, by 70% in comparison to controls (Clark et al., 1943). On the other hand, adding nutritional supplements of testosterone propionate increased the amount of pyruvic acid by 55% in normal mice, and restored the amount of pyruvic acid by 64% in castrated mice (Clark et al., 1943). This shows that increasing DAAO would consequently decrease the amount of D-serine, which implies sex-related differences in D-serine and, in turn, in NMDAR function where D-serine is the endogenous co-agonist. While the presence of DAAO has been detected in the retina (Romero et al., 2014), sex specific analysis was not performed, and male and female mice were used indiscriminately.

The main limitation to our work are that the exact concentration of D-serine in the retina is unknown. We chose to use topical delivery of D-serine to avoid puncturing the eye, which would activate signaling cascades involved NMDAR excitotoxicity, as it is well established that NMDAR is involved in the neurotoxicity of the retina (Bai et al., 2013). However, the data demonstrate that topical delivery of D-serine to the cornea reaches the retina at levels that affect significant changes in neuronal function; and this has never been documented before. Transport across the sclera and cornea have been studied for a number of molecules. Based on several studies, topical administration of small molecules

result in permeation through the cornea, and in even greater amounts, through the sclera (Cheruvu and Kompella, 2006; Pescina et al., 2015). In addition, the amino acid transporter ASCT1, shown to be specific for D-serine transport (Rosenberg et al., 2013) is found in the cornea (Katragadda et al., 2005). While the mechanism for uptake of topical D-serine from the cornea/scleral surface to the retina is unclear, its efficacy in replacing D-serine in the $SR^{-/-}$ mice demonstrates that it effectively reached the retina. Our future studies will use intravitreal injections of D-serine at different concentrations, and we will assess a- and b-wave amplitude and implicit time differences that may result from a more nuanced and localized exogenous delivery of D-serine. An in depth-analysis of the temporal properties of the a-wave in states of hypo and hyper-functionality of NMDAR is warranted. It is also apparent from this study that the fERG responses to stimuli need to encompass a wide range of temporal frequencies (temporal response function).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

1. Alteration in D-serine levels result in changes to the flash electroretinogram.
2. Reduced D-serine levels resulted in delayed a-waves and reduced b-waves.
3. These changes were sex-specific and only seen in male mice.
4. Gonadal hormones and D-serine play a role in retinal function integrity.

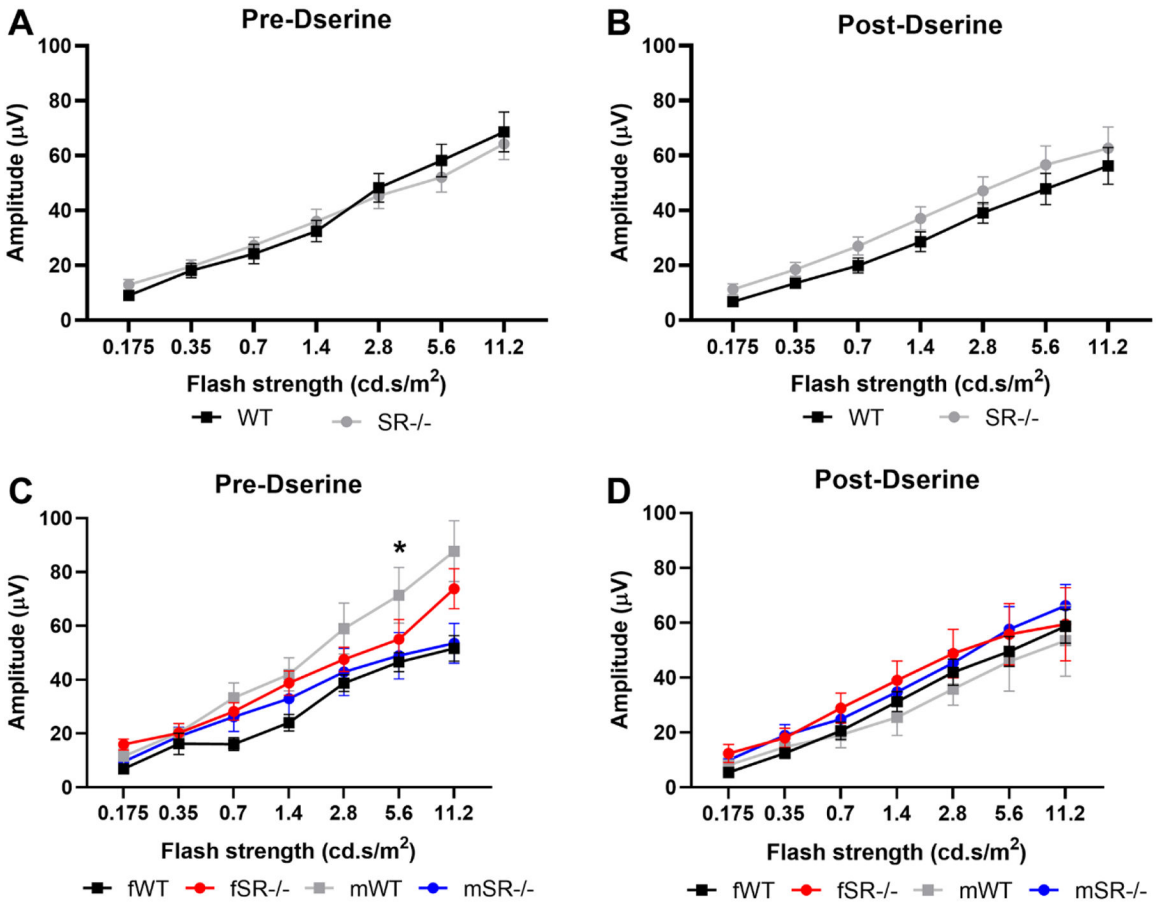

Figure 1. Effects of D-serine of the-wave amplitude.

(A) The a-wave amplitudes prior to treatment of all cohorts: female wild type (fWT), female serine racemase knockout ($fSR^{-/-}$), male wild type (mWT), and male serine racemase knockout ($mSR^{-/-}$). (B) The a-wave amplitudes after D-serine treatment for all cohorts. Data are expressed as mean +/− SEM. Asterisks indicate statistical significance. (C) The a-wave amplitudes prior to treatment separated based on sex. The only significant difference was between mWT at 5.6 cd.s/m^2. (D) The a-wave amplitudes post-D-serine treatment separated based on sex.

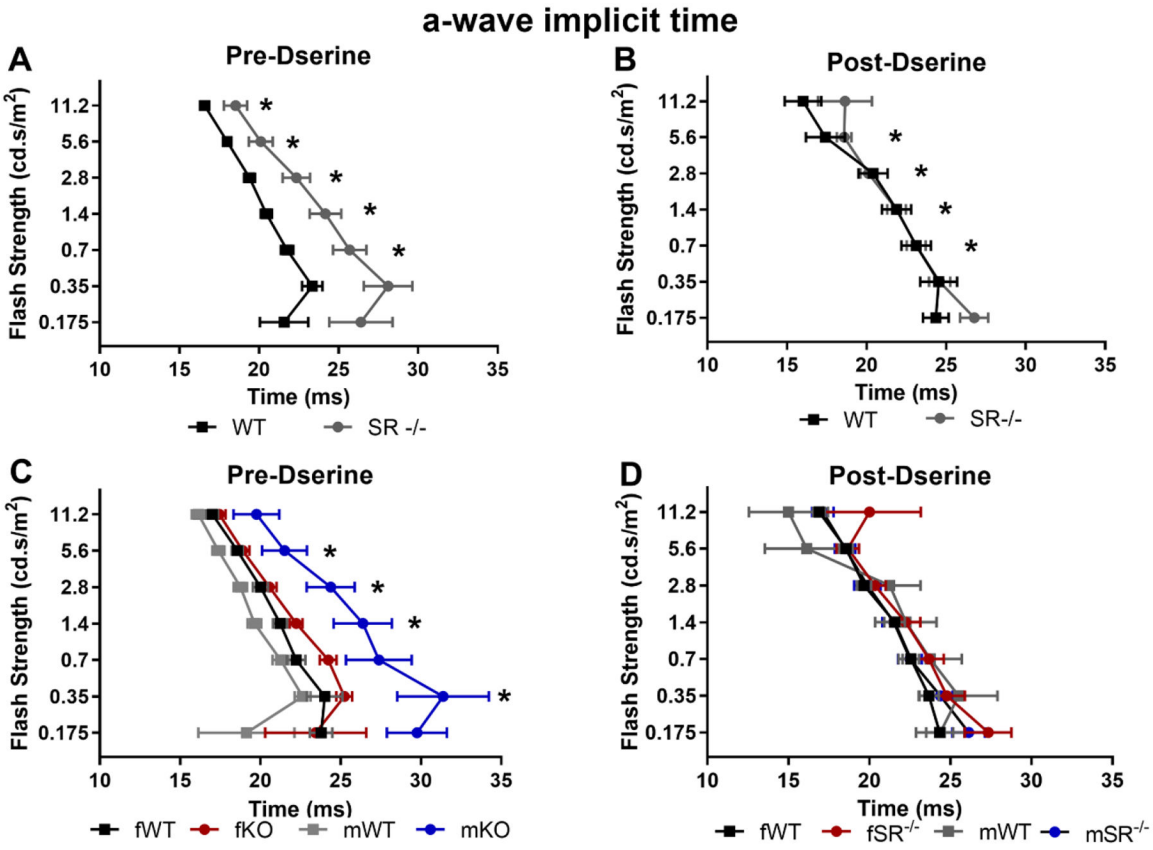

Figure 2. Effects of D-serine on the a-wave implicit time.

Comparison of the a-wave implicit time in WT and $SR^{-/-}$ mice before (A) and after (B) topical delivery of D-serine onto the cornea. There was a significant difference between the $SR^{-/-}$ mice and WT mice prior to D-serine treatment and a significant difference in the altered implicit time between the two genotypes. Significance in both A and B represents the interaction between treatment and genotype. Comparison between all groups, female wild type (fWT), female serine racemase knockout ($fSR^{-/-}$), male wild type (mWT), and male serine racemase knockout ($mSR^{-/-}$) before (C) or after (D) topical delivery of D-serine. Significance represents interaction between genotype and sex before D -serine (C) and after D-serine. Data are expressed as mean +/− SEM. Asterisks represent significant difference.

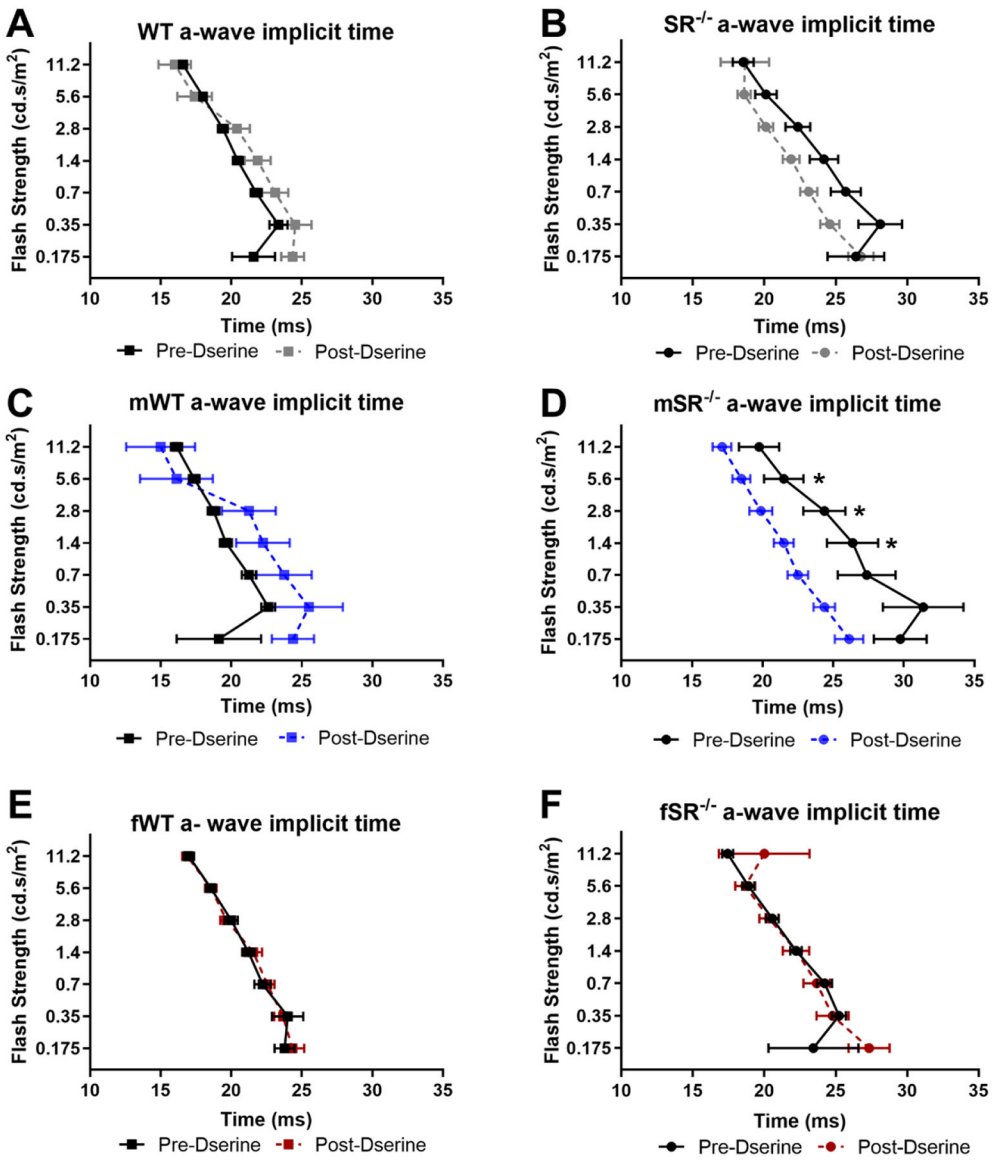

Figure 3. Effects of D-serine on a-wave implicit time: sex, genotype, and treatment.

The a-wave implicit time was not significantly different pre- and post-D-serine treatment. However, there was a significant delay in the a-wave implicit time in the male WT ERGs compared to the $SR^{-/-}$ controls. No differences were seen between female mice regardless of genotype or treatment. Asterisks indicate significant differences. Female wild type (fWT), female serine racemase knockout ($fSR^{-/-}$), male wild type (mWT), and male serine racemase knockout ($mSR^{-/-}$). Data are expressed as mean +/− SEM.

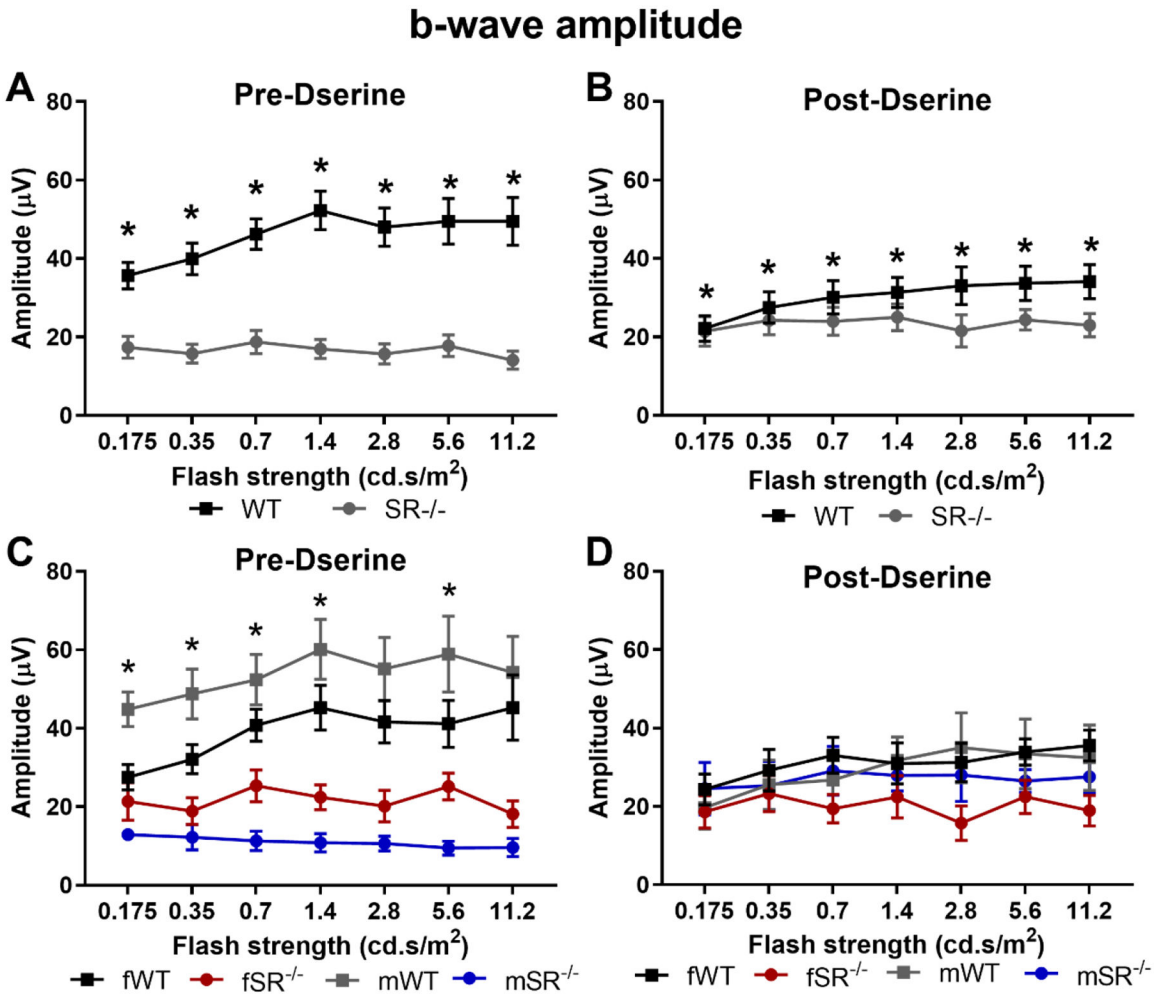

Figure 4. Effects of D-serine on b-wave amplitude.

Comparison of WT and $SR^{-/-}$ mice b-wave amplitude before (A) and after (B) topical delivery of D-serine. Significance in both A and B represent the interaction between treatment and genotype. Comparison between all groups, female WT (fWT), female $SR^{-/-}$ ($fSR^{-/-}$), male WT (mWT), and male $SR^{-/-}$ ($mSR^{-/-}$), before (C) or after (D) topical delivery of D-serine. Significance represents interaction between genotype and sex before D -serine (C) and after D-serine. Data are expressed as mean +/− SEM. Asterisks indicate statistical significance.

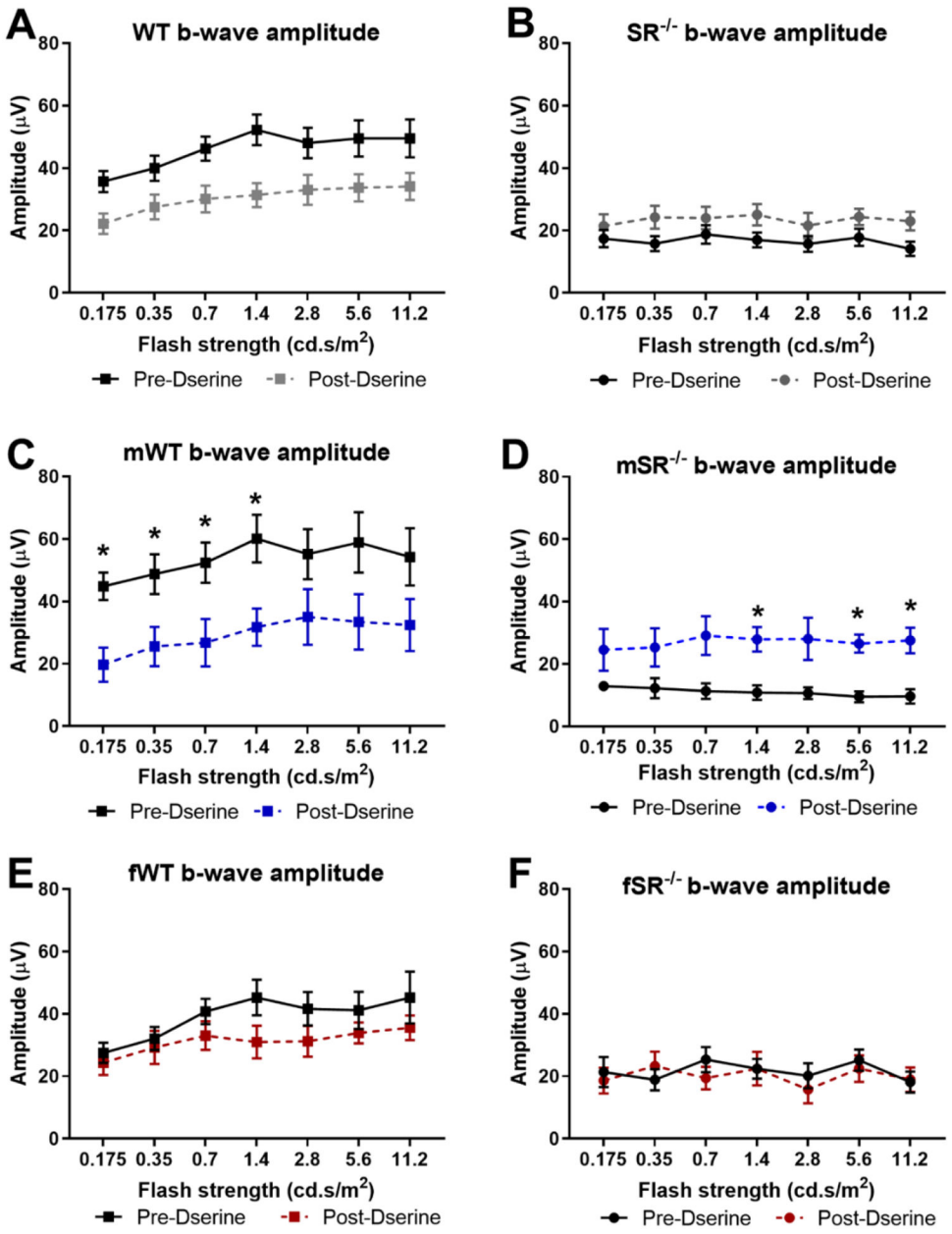

Figure 5. Effects of D-serine on the b-wave amplitude: sex, genotype, treatment.

In the male WT prior to treatment, the b-wave amplitude was significantly decreased compared to the WT controls, but only at the 4 lowest flash strengths. In the male $SR^{-/-}$ mice, D-serine treatment resulted in a significant increase in b-wave amplitude. No significant differences were seen in the female mice for either genotype or treatment. Data are expressed as mean +/− SEM. Asterisks indicate statistical significance.

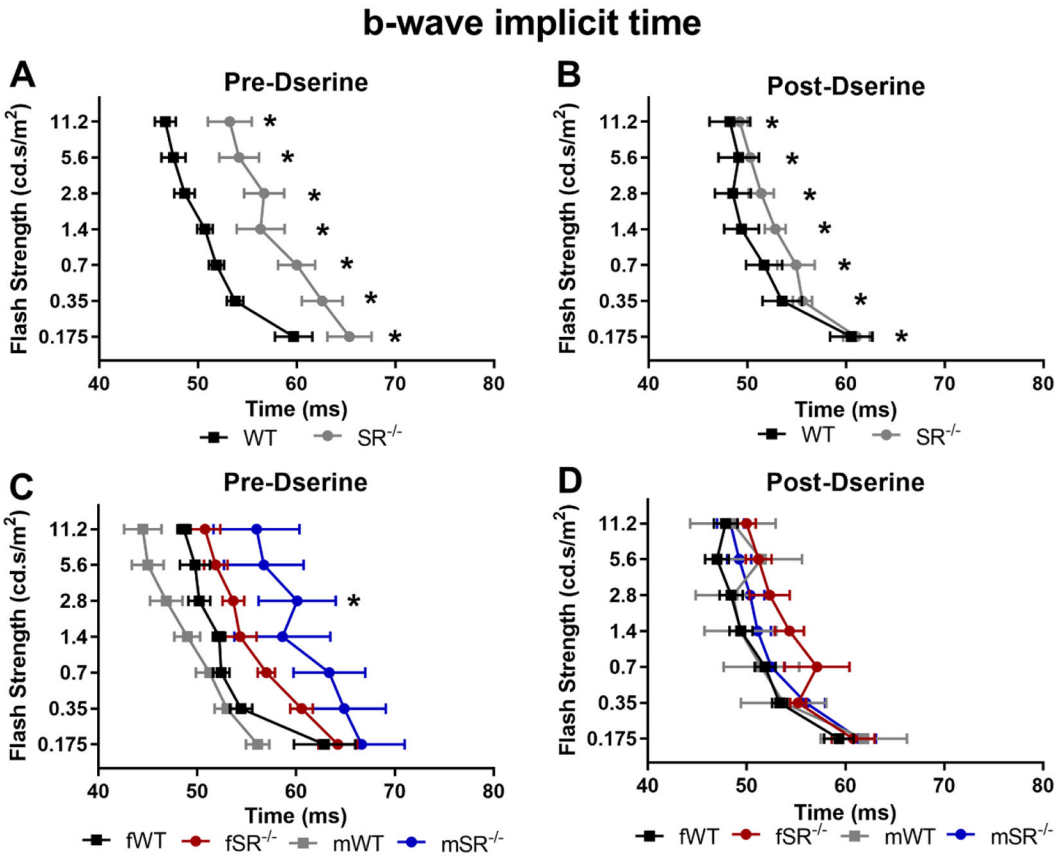

Figure 6. Effects of D-serine on b-wave implicit time.

Comparison of WT and $SR^{-/-}$ mice b-wave implicit time before (A) and after (B) topical delivery of D-serine. Significance in both A and B represent the interaction between treatment and genotype. Comparison between all groups, female WT (fWT), female $SR^{-/-}$ ($fSR^{-/-}$), male WT (mWT), and male $SR^{-/-}$ ($mSR^{-/-}$), before (C) or after (D) topical delivery of D-serine. Significance represents interaction between genotype and sex before D-serine (C) and after D-serine. Data are expressed as mean +/− SEM. Asterisks indicate statistical significance.

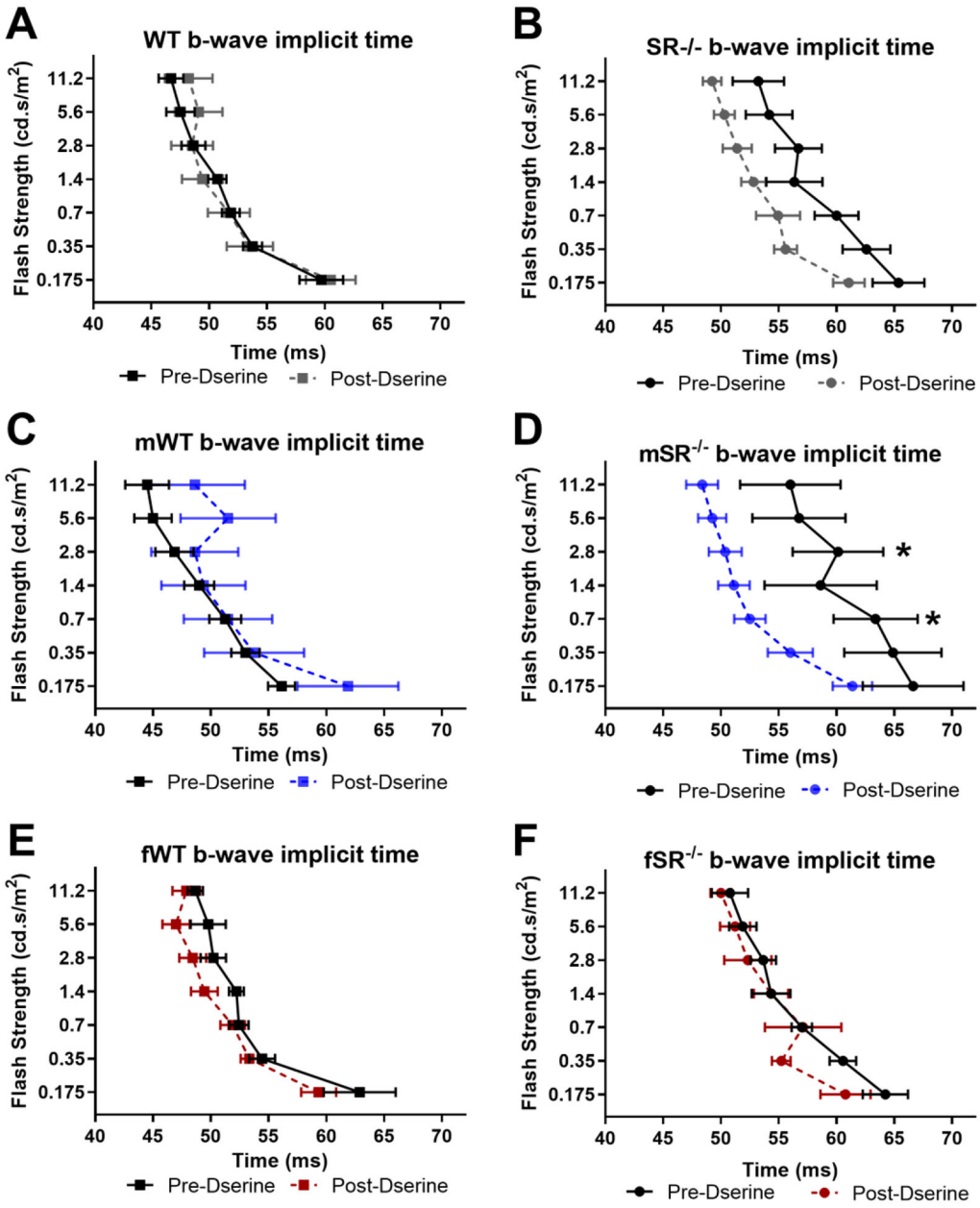

Figure 7. Effects of D-serine on b-wave implicit time: sex, genotype, and treatment.

Based on genotype or sex, very few significant differences were seen in the b-wave implicit times. There were several significant differences in the $SR^{-/-}$ mice, but overall there was a shift in the male $SR^{-/-}$ mice to a faster implicit time than in the absence of exogenously added D-serine.

Table 1.
Statistical analysis of the a-wave amplitude.

The top section shows the results of a three-way analysis for genotype, sex, and treatment. The bottom panel shows the results of an analysis of two-way interactions between treatment and genotype, treatment and sex, and pre- and post-treatment genotype and sex interactions. Red indicates statistical significance.

		a-wave amplitude	
		Treatment · Sex · Genotype	
Three-way interaction at each level of flash strength	**0.18**	F(1,30)=0.66 p =0.424	
	0.35	F(1,30)=0.17, p =0.683	
	0.7	F(1,30)=2.2, p =0.144	
	1.4	F(1,30)=3.0, p =0.095	
	2.8	F(1,30)=2.4, p =0.133	
	5.6	F(1,30)=2.4, p =0.136	
	11.2	F(1,30)=6.7, ***p* =0.015**	
		Treatment · Genotype	**Treatment · Sex**
Simple two-way interactions at each level of flash strength	**0.18**	F(1,30)=0.048, p =N.S.	F(1,30)=0.069, p = N.S.
	0.35	F(1,30)=0.576, p = N.S.	F(1,30)=0.0, p = N.S
	0.7	F(1,30)=0.643, p = N.S.	F(1,30)=3.4, p = N.S
	1.4	F(1,30)=0.602, p = N.S.	F(1,30)=2.3, p = N.S
	2.8	F(1,30)=1.7, p = N.S.	F(1,30)=2.0, p = N.S
	5.6	F(1,30)=1.8, p= N.S.	F(1,30)=0.7, p = N.S
	11.2	F(1,30)=9.4, p = N.S.	F(1,30)=0.296, p= N.S
		pre-Dser (Genotype · Sex)	**post-Dser (Genotype · Sex)**
	0.18	F(1,30)=5.43, p =0.027	F(1,30)=1.04, p = N.S.
	0.35	F(1,30)=0.55, p = N.S.	F(1,30)=0.05, p = N.S.
	0.7	F(1,30)=5.26, p =0.029	F(1,30)=0.08, p = N.S.
	1.4	F(1,30)=4.51, p =0.042	F(1,30)=0.02, p = N.S.
	2.8	F(1,30)=3.44, p = 0.073	F(1,30)=0.04, p = N.S.
	5.6	F(1,30)=4.10, p =0.052	F(1,30)=0.09, p = N.S.
	11.2	F(1,30)=12.88, ***p* = 0.001**	F(1,30)=0.32, p = N.S.

Table 2.

Analysis of a-wave amplitude pre- and post-D-serine for each cohort.

No significant differences were seen between any of the parameters analyzed.

Male a-wave amplitude								
	WT				***SR*** $^{-/-}$			
Flash strength (cd·s/m^2)	***Pre-Dserine***	***Post-Dserine***	***Mean Difference***	***p***	***Pre-Dserine***	***Post-Dserine***	***Mean Difference***	***p***
0.175	20 ± 3.7	8.1 ± 2.5	−3.4	N.S.	10 ± 2.4	9.9 ± 2.5	0.4	N.S.
0.35	49 ± 6.4	15 ± 3.2	−5.6	N.S.	19 ± 3.7	19 ± 3.2	0.0	N.S.
0.7	33 ± 4.3	19 ± 4.5	−14.2	N.S.	26 ± 4.3	25 ± 4.5	−1.3	N.S.
1.4	42 ± 5.8	26 ± 5.8	−16.5	N.S.	33 ± 5.8	35 ± 5.8	1.8	N.S.
2.8	59 ± 6.9	36 ± 6.6	−23.1	N.S.	43 ± 6.9	45 ± 6.6	2.5	N.S.
5.6	71 ± 7.9	46 ± 9.3	−25.5	N.S.	49 ± 7.9	57 ± 9.4	8.7	N.S.
11.2	**88 ± 8.1**	**53 ± 10.8**	−34.3	N.S.	54 ± 8.1	66 ± 10.2	12.7	N.S.
Female a-wave amplitude								
	WT				***SR*** $^{-/-}$			
0.175	7 ± 2.3	5.5 ± 2.4	0.6	N.S.	16 ± 2.3	12 ± 2.4	−3.6	N.S.
0.35	16 ± 3.5	12 ± 3.0	−0.3	N.S.	20 ± 3.5	18 ± 3.0	−2.1	N.S.
0.7	16 ± 4.1	21 ± 4.3	0.3	N.S.	28 ± 4.1	29 ± 4.3	0.6	N.S.
1.4	24 ± 5.4	31 ± 5.5	0.3	N.S.	39 ± 5.4	39 ± 5.3	6.0	N.S.
2.8	39 ± 6.5	42 ± 6.2	−0.3	N.S.	48 ± 6.5	49 ± 6.2	1.2	N.S.
5.6	47 ± 7.4	50 ± 8.9	0.0	N.S.	55 ± 7.4	56 ± 8.9	0.7	N.S.
11.2	52 ± 7.6	59 ± 10.2	−0.1	N.S.	74 ± 7.6	59 ± 10.2	−14.3	N.S.

Table 3.
Statistical analysis of a-wave implicit time.

There was a significant difference in a-wave implicit times between the $SR^{-/-}$ mouse and WT control mice, with the $SR^{-/-}$ showing a significant delay in the a-wave component. When analyzed for two-way interactions, there was a significant difference in a-wave implicit time for the majority of flash strengths, with the male $SR^{-/-}$ mice showing a significantly delayed implicit time compared to all other genotypes and between males and females. This difference was reduced by the D-serine treatment, causing a less delayed a-wave. Asterisks indicate significant difference.

		a-wave implicit time	
		Treatment · Sex · Genotype	
Three-way interaction at each level of flash strength	**0.18**	F(1,30)=6.75, ***p* = 0.014**	
	0.35	F(1,30)=6.28, ***p* =0.018**	
	0.7	F(1,30)=5.93, ***p* = 0.021**	
	1.4	F(1,30)=6.39, ***p* = 0.017**	
	2.8	F(1,30)=8.38, ***p* = 0.007**	
	5.6	F(1,30)=0.294, *p* = N.S.	
	11.2	F(1,30)=0.940, *p* = N.S.	
		Treatment · Genotype	**Treatment · Sex**
Simple two-way interactions at each level of flash strength	**0.18**	F(1,30)=1.39, *p* = N.S.	F(1,30)=0.36, *p* = N.S.
	0.35	F(1,30)=6.56, ***p* = 0.016**	F(1,30)=0.74, *p* = N.S.
	0.7	F(1,30)=9.62, ***p* = 0.004**	F(1,30)=0.65, *p* = N.S.
	1.4	F(1,30)=7.63, ***p* = 0.010**	F(1,30)=0.83, *p* = N.S.
	2.8	F(1,30)=7.86, ***p* = 0.009**	F(1,30)=0.35, *p* = N.S.
	5.6	F(1,30)=0.489, *p* =N.S.	F(1,30)=2.04, *p* = N.S.
	11.2	F(1,30)=0.074, *p* =N.S.	F(1,30)=2.07, *p* = N.S.
		pre-Dser (Genotype · Sex)	**post-Dser (Genotype · Sex)**
	0.18	F(1,30)=5.28, *p* =0.029	F(1,30)=0.3, p= N.S.
	0.35	F(1,30)=6.2, ***p* =0.018**	F(1,30)=0.7, p= N.S.
	0.7	F(1,30)=3.70, *p* = N.S.	F(1,30)=1.1, p= N.S.
	1.4	F(1,30)=9.60, ***p* =0.004**	F(1,30)=0.4, p= N.S.
	2.8	F(1,30)=9.86, ***p* =0.004**	F(1,30)=0.9, p= N.S.
	5.6	F(1,30)=6.57, ***p* =0.016**	F(1,30)=0.7, p= N.S.
	11.2	F(1,30)=4.4, *p* =0.042	F(1,30)=0.6, p= N.S.

Table 4.
Effect of D-serine on the a-wave implicit time for each cohort.

Analysis of a-wave implicit time shows significant differences based on D-serine treatment in the male SR−/− mice, but only at the middle range of flash strengths. No significant differences were seen as a result of treatment in either the male or female WT mice. Similarly, there were no significant differences in a-wave implicit time in the female SR−/− mice at any of the flash strengths.

Male a-wave implicit time								
	WT				***SR*** $^{-/-}$			
Flash strength (cd·s/m^2)	***Pre-Dserine***	***Post-Dserine***	***Mean Difference***	***p***	***Pre-Dserine***	***Post-Dserine***	***Mean Difference***	***p***
0.175	19 ± 2.5	24 ± 1.3	5.3	N.S.	30 ± 2.5	26 ± 1.3	−3.6	N.S.
0.35	23 ± 1.5	26 ± 1.4	2.3	N.S.	31 ± 1.5	25 ± 1.4	−7.0	0.036
0.7	21 ± 1.1	24 ± 1.2	2.5	N.S.	27 ± 1.1	23 ± 1.2	−4.9	**0.011**
1.4	20 ± 1.0	22 ± 1.2	2.6	N.S.	26 ± 1.0	22 ± 1.2	−4.9	**0.023**
2.8	19 ± 0.8	21 ± 1.1	2.5	N.S.	24 ± 0.8	20 ± 1.1	−4.5	**0.013**
5.6	17 ± 0.8	16 ± 1.4	1.3	N.S.	22 ± 0.8	19 ± 1.4	−3.0	N.S.
11.2	16 ± 0.8	15 ± 2.4	1.1	N.S.	20 ± 0.8	17 ± 2.1	−2.6	N.S.
Female a-wave implicit time								
	WT				***SR*** $^{-/-}$			
0.175	24 ± 2.3	24 ± 1.2	0.6	N.S.	23 ± 2.3	27 ± 1.2	3.9	N.S.
0.35	24 ± 1.5	24 ± 1.3	−0.3	N.S.	25 ± 1.5	25 ± 1.3	−0.4	N.S.
0.7	22 ± 1.0	23 ± 1.1	0.3	N.S.	24 ± 1.0	24 ± 1.1	−0.6	N.S.
1.4	21 ± 1.0	22 ± 1.1	0.3	N.S.	22 ± 1.0	22 ± 1.1	0.0	N.S.
2.8	20 ± 0.8	20 ± 1.0	−0.3	N.S.	21 ± 0.8	20 ± 1.0	−0.2	N.S.
5.6	19 ± 0.7	19 ± 1.3	0.0	N.S.	19 ± 0.7	19 ± 1.3	−0.2	N.S.
11.2	17 ± 0.7	17 ± 2.0	−1.1	N.S.	17 ± 0.7	20 ± 2.0	−2.6	N.S.

Table 5.
Statistical analysis of the b-wave amplitude.

There was a statistically significant reduction in b-wave amplitude of the $Sr^{-/-}$ mice compared to WT controls at all flash strengths. When examined for interactions of sex and genotype, there again was a significant difference between male WT and male $SR^{-/-}$ mice, with the male SR−/− mice showing a significant reduction in b-wave amplitude compared to the male WT controls. After D-serine treatment, there were no significant differences in b-wave amplitude between any of the cohorts. Asterisks indicate significant difference. Female wild type (fWT), female serine racemase knockout ($fSR^{-/-}$), male wild type (mWT), and male serine racemase knockout ($mSR^{-/-}$).

		b-wave amplitude	
		Treatment · Sex · Genotype	
Three-way interaction at each level of flash strength	**0.18**	F(1,30)=9.31, ***p* =0.005**	
	0.35	F(1,30)=4.55, ***p* =0.041**	
	0.7	F(1,30)=11.05, ***p* =0.002**	
	1.4	F(1,30)=11.05, ***p* =0.002**	
	2.8	F(1,30)=4.77, ***p* =0.037**	
	5.6	F(1,30)=10.88, ***p* =0.003**	
	11.2	F(1,30)=4.93, ***p* =0.034**	
		Treatment · Genotype	**Treatment · Sex**
Simple two-way interactions at each level of flash strength	**0.18**	F(1,30)=9.73, ***p* =0.004**	F(1,30)=0.41, *p* = N.S.
	0.35	F(1,30)=10.26, ***p* =0.003**	F(1,30)=0.74, *p* = N.S.
	0.7	F(1,30)=13.08, ***p* =0.001**	F(1,30)=0.22, *p* = N.S.
	1.4	F(1,30)=22.64, ***p*<0.0005**	F(1,30)=0.05, *p* = N.S.
	2.8	F(1,30)=9.07, ***p* =0.005**	F(1,30)=0.71, *p* = N.S.
	5.6	F(1,30)=16.74, ***p*<0.0005**	F(1,30)=0.02, *p* = N.S.
	11.2	F(1,30)=14,53, ***p* =0.001**	F(1,30)=0.14, *p* = N.S.
		pre-Dser (Genotype · Sex)	**post-Dser (Genotype · Sex)**
	0.18	F(1,30)=11.8, ***p* =0.002**	F(1,30)=1.1 *p* = N.S.
	0.35	F(1,30)=7.4, ***p* =0.011**	F(1,30)=0.26, *p* = N.S.
	0.7	F(1,30)=8.3, ***p* =0.007**	F(1,30)=2.1, *p* = N.S.
	1.4	F(1,30)=6.7, ***p* =0.015**	F(1,30)=0.20, *p* = N.S.
	2.8	F(1,30)=4.8, *p* = 0.036	F(1,30)=0.46, *p* = N.S.
	5.6	F(1,30)=8.1, ***p* =0.008**	F(1,30)=0.19, *p* = N.S.
	11.2	F(1,30)=1.8, *p* = N.S.	F(1,30)=1.2, *p* = N.S.

Table 6.

Effect of D-serine on b-wave amplitude for each group.

Analysis of the b-wave amplitude for male and female mice. Only the male mice showed significant changes in b-wave amplitude, both pre-D-serine treatment and post-D-serine treatment. Red indicates significance.

Male b-wave amplitude								
	WT				***SR*** $^{-/-}$			
Flash intensity (cd·s/m^2)	***Pre-Dserine***	***Post-Dserine***	***Mean Difference***	***p***	***Pre-Dserine***	***Post-Dserine***	***Mean Difference***	***p***
0.175	45 ± 4.4	20 ± 5.4	−25.4	**0.002**	13 ± 1.2	24 ± 5.4	11.6	0.152
0.35	49 ± 6.4	26 ± 6.3	−23.2	**0.008**	12 ± 3.2	25 ± 6.1	13.1	0.102
0.7	52 ± 6.4	27 ± 7.6	−25.6	**0.009**	11 ± 2.5	29 ± 6.2	17.8	0.302
1.4	60 ± 7.6	32 ± 6.0	−28.4	**0.003**	11 ± 2.3	28 ± 3.9	17.1	**0.021**
2.8	55 ± 8.0	35 ± 8.9	−20.1	0.085	11 ± 1.9	28 ± 6.8	17.5	0.043
5.6	59 ± 9.7	33 ± 8.9	−25.5	0.036	9 ± 1.8	27 ± 2.9	17.0	**0.004**
11.2	54 ± 9.2	32 ± 8.3	−21.8	0.035	10 ± 2.3	28 ± 4.1	17.9	**0.020**
Female b-wave amplitude								
	WT				***SR*** $^{-/-}$			
0.175	28 ± 3.2	24 ± 3.9	−3.2	0.564	21 ± 4.8	19 ± 4.2	−2.8	0.661
0.35	32 ± 3.7	29 ± 5.2	−2.9	0.664	19 ± 3.4	23 ± 4.6	4.4	0.508
0.7	41 ± 4.1	33 ± 4.6	−7.7	0.134	25 ± 4.0	19 ± 3.6	−8.9	0.377
1.4	45 ± 5.7	31 ± 5.3	−14.3	0.031	22 ± 3.2	22 ± 5.3	0.1	0.994
2.8	42 ± 5.4	31 ± 4.9	−10.4	0.037	20 ± 4.0	16 ± 4.4	−4.3	0.546
5.6	41 ± 6.0	34 ± 3.3	−7.3	0.079	25 ± 3.4	22 ± 4.2	−2.7	0.513
11.2	45 ± 8.3	36 ± 3.9	−9.7	0.195	18 ± 3.3	19 ± 3.9	0.8	0.876

Table 7.
Statistical analysis of b-wave implicit time.

An analysis for a three-way interaction for treatment, sex, and genotype showed that there was a significant difference between the groups. A two-way interaction analysis showed that for treatment and genotype, the male mice showed a significantly increased b-wave implicit time.

		b-wave implicit time	
		Treatment · Sex · Genotype	
Three-way interaction at each level of flash strength	**0.18**	F(1,30)=9.31, ***p* =0.005**	
	0.35	F(1,30)=6.28, ***p* =0.018**	
	0.7	F(1,30)=11.05, ***p* =0.002**	
	1.4	F(1,30)=11.05, ***p* =0.002**	
	2.8	F(1,30)=4.77, ***p* =0.037**	
	5.6	F(1,30)=10.88, ***p* =0.003**	
	11.2	F(1,30)=4.93, ***p* =0.034**	
		Treatment · Genotype	**Treatment · Sex**
Simple two-way interactions at each level of flash strength	**0.18**	F(1,30)=9.73, ***p* =0.004**	F(1,30)=0.41, *p* = N.S.
	0.35	F(1,30)=10.26, ***p* =0.003**	F(1,30)=0.74, *p* = N.S.
	0.7	F(1,30)=13.08, ***p* =0.001**	F(1,30)=0.22, *p* = N.S.
	1.4	F(1,30)=22.64, ***p*< 0.0005**	F(1,30)=0.05, *p* = N.S.
	2.8	F(1,30)=9.07, ***p* =0.005**	F(1,30)=0.71, *p* = N.S.
	5.6	F(1,30)=16.74, ***p*< 0.0005**	F(1,30)=0.02, *p* = N.S.
	11.2	F(1,30)=14.53, ***p* =0.001**	F(1,30)=0.14, *p* = N.S.
		pre-Dser (Genotype · Sex)	**post-Dser (Genotype · Sex)**
	0.18	F(1,30)=2.5, *p* = N.S.	F(1,30)=0.1, p= N.S.
	0.35	F(1,30)=1.7, *p* = N.S.	F(1,30)=0.0, p= N.S.
	0.7	F(1,30)=3.8, *p* = N.S.	F(1,30)=0.6, p= N.S.
	1.4	F(1,30)=2.2, *p* = N.S.	F(1,30)=0.6, p= N.S.
	2.8	F(1,30)=5.2, ***p* = 0.03**	F(1,30)=0.2, p= N.S.
	5.6	F(1,30)=4.5, *p* = N.S.	F(1,30)=2.1, p= N.S.
	11.2	F(1,30)=3.8, *p* = N.S.	F(1,30)=0.3, p= N.S.

Table 8.
Effect of D-serine on the b-wave implicit time for each group.

Relative to genotype and treatment, there were relatively few significant differences in the b-wave implicit time before or after D-serine, with only significant differences seen at two flash strengths based genotype in the male mice and one based on genotype in the female mice. Red indicates statistical significance.

Male b-wave implicit time								
	WT				***SR*** $^{-/-}$			
Flash strength (cd·s/m^2)	***Pre-Dserine***	***Post-Dserine***	***Mean Difference***	***p***	***Pre-Dserine***	***Post-Dserine***	***Mean Difference***	***p***
0.175	56 ± 3.0	62 ± 2.7	5.8	N.S.	67 ± 3.0	61 ± 2.7	−5.3	N.S.
0.35	53 ± 2.3	54 ± 2.4	0.8	N.S.	65 ± 2.3	56 ± 2.4	−8.9	N.S.
0.7	51 ± 2.0	52 ± 2.8	0.3	N.S.	63 ± 2.0	53 ± 2.8	−10.9	**0.018**
1.4	49 ± 2.6	49 ± 2.1	0.4	N.S.	59 ± 2.6	51 ± 2.1	−7.5	N.S.
2.8	47 ± 2.2	49 ± 2.3	1.8	N.S.	60 ± 2.2	50 ± 2.3	−9.8	**0.021**
5.6	45 ± 2.3	52 ± 2.3	6.5	N.S.	57 ± 2.3	49 ± 2.3	−7.5	N.S.
11.2	45 ± 2.5	49 ± 2.3	4.1	N.S.	56 ± 2.5	48 ± 2.3	−7.6	N.S.
Female b-wave implicit time								
	WT				***SR*** $^{-/-}$			
0.175	63 ± 2.8	59 ± 2.5	−3.6	N.S.	64 ± 2.8	61 ± 2.5	−3.4	N.S.
0.35	54 ± 2.2	53 ± 2.2	−1.1	N.S.	61 ± 2.2	55 ± 2.2	−5.3	**0.005**
0.7	52 ± 1.9	52 ± 2.6	−0.6	N.S.	57 ± 1.9	57 ± 2.6	0.1	N.S.
1.4	52 ± 2.5	49 ± 2.0	−2.8	N.S.	54 ± 2.5	54 ± 2.0	0.0	N.S.
2.8	50 ± 2.1	48 ± 2.2	−1.8	N.S.	54 ± 2.1	52 ± 2.2	−1.3	N.S.
5.6	50 ± 2.2	47 ± 2.2	−2.8	N.S.	52 ± 2.2	51 ± 2.2	−0.7	N.S.
11.2	49 ± 2.3	48 ± 2.2	−0.8	N.S.	51 ± 2.3	50 ± 2.2	−0.8	N.S.