Accepted Manuscript

Title: The Role of Ephrin-A2 and Ephrin-A5 in Sensorimotor Control and Gating

Author: Nathanael J. Yates Mathew T. Martin-Iverson
Jennifer Rodger

PII: S0166-4328(14)00580-4
DOI: <http://dx.doi.org/doi:10.1016/j.bbr.2014.08.061>
Reference: BBR 9135

To appear in: *Behavioural Brain Research*

Received date: 14-7-2014
Revised date: 28-8-2014
Accepted date: 30-8-2014

Please cite this article as: Yates NJ, Martin-Iverson MT, Rodger J, The Role of Ephrin-A2 and Ephrin-A5 in Sensorimotor Control and Gating, *Behavioural Brain Research* (2014), <http://dx.doi.org/10.1016/j.bbr.2014.08.061>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

- The acoustic startle reflex can detect changes in sensorimotor gating and function
- Ephrin-A2 and ephrin-A5 are neurodevelopmental proteins expressed in this pathway
- We examined the role of ephrin-A2 and ephrin-A5 KO on the acoustic startle reflex
- Ephrin-A2 KO increases auditory sensitivity and motor function, ephrin-A5 decreases
- Neither ephrin-A2 or ephrin-A5 affected sensorimotor gating

The Role of Ephrin-A2 and Ephrin-A5 in Sensorimotor Control and Gating

Nathanael J. Yates 1, 2, 3, Mathew T. Martin-Iverson 3, Jennifer Rodger 1

Schools of Animal Biology (1), Anatomy, Physiology and Human Biology (2), School of
Medicine and Pharmacology (3), The University of Western Australia, 35 Stirling Highway,
Crawley WA 6009, Australia

Word count

Introduction: 724

Discussion: 1,815

Author for correspondence:

Nathanael Yates

Experimental and Regenerative Neuroscience

School of Animal Biology M317

University of Western Australia

35 Stirling Highway

Crawley WA 6009

Australia

Tel: 618 6488 3524

email: nathanael.yates@research.uwa.edu.au

Keywords: ephrin; prepulse inhibition; startle reflex; dopamine; muscle; hearing

Abstract

Many factors influence neurodevelopment. However, their contribution to adult neural function is often unclear. This is often due to complex expression profiles, cell signalling, neuroanatomy, and a lack of effective tests to assess the function of neural circuits *in vivo*. Ephrin-A2 and ephrin-A5 are cell surface proteins implicated in multiple aspects of neurodevelopment. While the role of ephrin-As in visual, auditory and learning behaviours has been explored, little is known about their role in dopaminergic and neuromotor pathways, despite expression in associated brain regions. Here we probe the function of ephrin-A2 and ephrin-A5 in the development of the dopaminergic and neuromotor pathways using counts of tyrosine hydroxylase (TH) positive cells in the substantia nigra pars compacta (SNpc) and the ventral tegmental area (VTA), the acoustic startle reflex (ASR), and a measure of sensorimotor gating, prepulse inhibition (PPI). Analysis of the ASR and PPI in ephrin-A2 and/or ephrin-A5 knock-out mice revealed that both genes play distinct roles in mediating ASR circuits, but are unlikely to play a role in PPI. Knock-out of either gene resulted in robust changes in startle response magnitude and measures of startle onset and peak latencies. However, ephrin-A2 and ephrin-A5 regulate aspects of the ASR differently: ephrin-A2 KO mice have increased startle amplitude, increased sensitivity and reduced latency to startle, whilst ephrin-A5 KO mice show opposite effects. Neither of the gene knock outs affected PPI, despite ephrin-A5 KO mice showing changes in dopamine cell numbers in nuclei thought to regulate PPI. We propose that majority of the changes observed ephrin-A2 and ephrin-A5 KO mice appear to be mediated by the effects on motor neurons and their muscle targets, rather than changes in auditory sensitivity.

Introduction

Many factors influence neurodevelopment; however, their contribution to adult neural function is often unclear. This is often due to complex expression profiles, cell signalling, neuroanatomy, and a lack of effective tests to assess the function of neural circuits *in vivo*. The use of knock-out models, however, has been advantageous in this regard. Ephrin-A2 and ephrin-A5 are cell surface proteins, which are implicated in multiple aspects of neurodevelopment including axonal guidance [1, 2] and neurogenesis [3, 4] in many brain regions. While the roles of ephrin-As in visual, auditory and learning behaviours have been explored, little is known about their role in dopaminergic and neuromotor pathways despite expression in associated brain regions (dopamine: [5-7]; neuromotor: [8-11]). Furthermore, these pathways are highly suited for functional assessments because of the well-defined combination of reflexes, sensorimotor processes such as the acoustic startle reflex (ASR), and modulation of the ASR by prepulse inhibition (PPI). ASR and PPI together have been probed in many animal studies which primarily examine the role of the dopamine system, due to elevated dopamine levels in these pathways being associated with disrupted PPI and symptoms of schizophrenia [12-16].

The acoustic startle response (ASR) is a reflex with well-characterised neuroanatomy that is observed in many species. The reflex occurs when a loud and sudden sound stimulus [17] produces a widespread musculoskeletal response comprising of a variety of protective behaviours [18]. The primary ASR pathway is a relatively simple short-latency circuit originating in the cochlea, proceeding through the lower brainstem, to cranial and spinal motor nuclei [19]. The size of the primary ASR is dependent on several factors such as auditory sensitivity [20-22], age [23], species and strain [23-25], weight, habituation [26], attention [27] and emotion [14, 28]. The ASR circuit encompasses many regions that express ephrin-A2 and ephrin-A5, including the afferent sensory portions, the cochlea [29-32] and auditory nerve

[33, 34], where they appear to have an important role in hearing development [35]. Ephrin-A2 and ephrin-A5 are also expressed in the efferent neuromotor portion of the ASR circuit. They are expressed in dorsal root ganglion cells and in motor neurons that project to the hindlimb [10]. The ASR appears to be excellent method to assess the role of ephrin-A2 and ephrin-A5 proteins in these regions due to its simple and well-defined neuroanatomical substrates.

The ASR response itself can be modulated or ‘gated’ by other stimuli, providing information on sensory, motor, and dopamine-mediated processing. This form of sensorimotor gating can be observed in the PPI paradigm. This occurs when the ASR response is inhibited by smaller non-startling stimulus, called the prepulse, that shortly precedes the startling stimulus. PPI of the ASR reflects subcortical attentive processing [36] and is modulated by several ascending and descending pathways. These pathways include the dopaminergic mesolimbic pathway [37], which originates in the ventral tegmental area (VTA) and terminates in the nucleus accumbens [38]. Expression and neuroanatomical data supports a potential role for ephrin-A2 and ephrin-A5 in the dopamine pathways, and potentially PPI. Ephrin-A2 and ephrin-A5 are expressed in the nucleus accumbens a major terminal area of dopamine neurons, and regulator of PPI [5-7, online Allen Mouse Brain Atlas, 39]. Furthermore ephrin-A2 and ephrin-A5 expressing neurons are capable of interacting with dopaminergic EphA5-expressing neurons from the VTA [5, 7, 40]. There is evidence for the involvement of ephrin-A5 in the dopaminergic system. Ephrin-A5 regulates the formation of ascending dopaminergic midbrain projections [7] and acts as a repulsive cue to dopaminergic neurons in culture stripe-assays [5]. Furthermore, the knock-out of ephrin-A5 results in reductions in of dopamine levels and increases in DOPAC levels (a dopamine metabolite) in the striatum, suggesting an increase in dopamine turnover [41].

Our study utilized an ASR paradigm to assess the potential role of ephrin-A2 and ephrin-A5 in regulating sensorimotor control and gating in knock-out mouse models. We used the properties of the ASR and PPI to probe the unique contribution of ephrin genes to the functional neurodevelopment of auditory, dopamine, and motor pathways. Our results suggest that ephrin-A2 and ephrin-A5 affect the motor response component of the ASR, and that ephrin-A5 may play a role in regulating the development of dopamine midbrain nuclei. In particular there is evidence suggesting that ephrin-A2 and ephrin-A5 have an important role in neuro-motor development, but with each gene exerting opposite effects. However, neither ephrin-A2 nor ephrin-A5 have a major role in regulating sensorimotor gating.

Methods:

Animals

Four genotypes were used in this study, Ephrin-A2^{-/-} (N=11), Ephrin-A5^{-/-} (N=11), Ephrin-A2A5^{-/-} (N=10) and wild-type (WT, N=13). All animals were male adults with a background strain of C57BL/6J and were younger than onset age of presbycusis for this strain (all animals <35 weeks old, [42, 43]. Animals were housed in standard cages (45cm x 29 cm x 12 cm), 12 hour light/dark cycle, and standard enrichment conditions. They were housed in groups until 1 week before testing, when they were housed individually.

Equipment and Startle Paradigm

All animals were habituated to the holding chambers, handling, and the experimental room for a week prior to the experiment. The mice were habituated to the experiment by going through one startle testing run before the experimental day shown in this study. For the PPI experiments all mice were placed in transparent Perspex holding cages and placed in sound-attenuated chambers equipped with speakers, ventilation fans and startle platforms (MEDAssociates, Software :SOF-815 Version 5.0; Hardware: ANL-925D, ANL-925E and ANL-729, cages: ENV-263A).

The PPI conditions were as follows: Background uniform white noise level 70 dB SPL, 8 ms broadband noise prepulse (75 dB SPL, 1 ms rise/fall time), beginning 50 ms prior to the onset of a startle pulse (40 ms duration broad-band noise startle stimuli with 0 ms rise/fall time and between 70 and 120 dB SPL in 5 dB increments, but presented randomly within the set of 11 intensities). The inter-trial interval was randomly chosen for each trial between 10 and 15 s.

At the beginning of each day the sound stimuli were calibrated using the MEDAssociates ANL-929A-PC USB SPL measurement package. There were 4 blocks of trials and each block consisted of 11 prepulse + pulse trial with one each of the pulse intensities and another

11 pulse trials with one each of the pulse intensities but no prepulses. Each stimulus combination, with and without prepulse, was presented in pseudorandom order in each block, but this order was kept the same for each animal. The response to each combination of stimuli was averaged across the 4 blocks. The individual trial startle magnitude was taken to be the area under the curve of the largest peak amplitude of the startle response. The study design allowed for assessment of stimulus intensity-response magnitude (SIRM) relationships.

Analysis of ASR and PPI

The presentation of a variety of startle pulse intensities allows for unique component measures of the startle response and sensorimotor gating to be assessed [25, 27, 44]. A curve of best fit was calculated using nonlinear regression in R statistics (version 3.0.1) with the "nls" function of the Package *stats* (version 3.0.1) and the port algorithm for iterative estimation for each animal and prepulse condition. The startle response curve was modelled as below as described by Scholes and Martin-Iverson [27]:

$$\text{Eq. (1)} \quad y = R_{max} + \frac{(y_0 - R_{max})}{1 + \left(\frac{x}{ES_{50}}\right)^{Hillslope}};$$

Where y = startle response magnitude, x = startle pulse intensity in dB. R_{MAX} = startle maximum asymptote predicted by the curve, y_0 = y-axis intercept. ES_{50} is the fitted value SPL (dB) where the response is predicted to be half of maximum. Hillslope = maximum velocity where acceleration is 0 (i.e., the rate of change at the ES_{50}). The following constraints were placed on the equation fit: $0.5 \times \text{Maximum Startle} \leq R_{MAX} \leq 1.25 \times \text{Maximum Startle}$, $0 \leq y_0 \leq \text{Maximum Startle}$, $71 \leq ES_{50} \leq 121$, and $Hillslope \geq 0$.

The equation for calculation of startle threshold is shown below in Eq. (2). Unlike over parameters, threshold estimates were derived from curve-fits using all response magnitudes expressed as a percent of maximum wild-type response. This is because estimates of threshold are inaccurate with large R_{max} and y_0 values ($>>100$ units).

$$\text{Eq. (2) } Threshold = ES_{50} - \frac{R_{max} - y_0}{Hillslope}$$

The calculation of percent prepulse inhibition of R_{max} was performed using Eq. (3).

$$\text{Eq. (3) } \%PPI = \frac{R_{maxStartle} - R_{maxprepulse}}{R_{maxStartle}} \times 100$$

Statistical analysis was performed in R studio (version 0.97.551) and R (version 3.0.1), with packages ez (version 4.1-1) and plyr (version 1.8). Data were analysed with a 2-factor ANCOVA design, with ephrin-A2 genotype and ephrin-A5 genotype as independent factors, each with two levels (KO and wild-type) and between-subjects covariates of age and body mass. An $\alpha = 0.05$ was used for the study as the level of significance. Because each factor only had 2 levels in the design of the analysis, post-hoc comparisons were only used when a significant interaction term was found. When this was the case Holm-Bonferroni exact corrected t-tests were used. Analyses within figures are presented as differences due to genotypes. They are labelled with A2^{KO} and A5^{KO} to represent differences due to ephrin-A2 and ephrin-A5 genotypes respectively, followed by information in brackets the columns of the figure panel that are being compared. When interactions are present, they are labelled as A2^{KO} vs. A5^{KO}.

For most analyses, the parameters from the curve fitting were the dependent variables as described above (R_{MAX} , ES_{50} , Hillslope and threshold), with the startle pulse alone trials analysed separately from the trial that had a prepulse present. All results were tested for assumptions of homogeneity of variance using Levene's test or assumptions of sphericity using Mauchly's test of Sphericity, where relevant.

Immunohistochemistry

Three wild type mice and five of each of the other genotypes were randomly selected for immunohistochemistry and after anaesthesia were perfused with saline followed by 4% paraformaldehyde. Brains were removed and left in 30% sucrose in PBS overnight. The brains were then cut on a cryostat at 40 μ m with alternate sections placed on slides or in 24 well plates. The staining for tyrosine hydroxylase (TH), a marker of catecholaminergic cells, was done as follows. 2x PBS wash, 20 min incubation in 0.3% H₂O₂ in methanol, wash in 0.2% Triton X-100 in PBS, 2x PBS wash and 72 hours incubation in 1:20 000 mouse monoclonal anti-tyrosine hydroxylase (Sigma-Aldrich, T2928), 10% horse serum, 0.2% BSA, 0.3% Triton X-100 agitating at 4° C. This was followed by 3x PBS washes, 30 min wash in 0.2% BSA, 10% Horse Serum, 90 min in 1:1000 biotinylated horse anti-mouse (Vectastain ABC Kit, BA-2000, Vector Laboratories) 0.2% BSA, 10% Horse Serum, 3x PBS Wash, 120 min 1:400 ABC solution (1:400 A and 1:400 B (Vectastain ABC Kit, PK-4002, Vector Laboratories), made in PBS at least 30 min before use), 3x PBS Wash, DAB solution for 4 min, 3x PBS wash, mount and allow to dry, dehydrated and cleared, and then coverslipped with Entellan. The slides that were not used for TH staining were stained using cresyl violet and were used as reference material for anatomical verification. All slides were scanned at 20x magnification (ScanScope XT, Aperio), and every second section of the TH (every fourth section in total) was examined for numbers of TH⁺ cells in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNpc), and the areas of these nuclei based upon Fu et al. [45]. Cells were counted only if the nucleus was visible in the focal plane, and VTA and SNpc areas were based upon the area within the boundaries of TH⁺ cells.

Results

Following each experiment, the average responses at each stimulus intensity for the pulse alone trials and separately for the prepulse + pulse trials were fitted as described to Eq. 1, generating stimulus intensity-response magnitude (SIRM) functions and derived parameters as described above. The latency of the startle responses from the onset of the startle pulses was also recorded.

Acoustic Startle Reflex

Startle response magnitudes

The major outcome measure of startle reactivity in our mice was their startle response magnitudes, an indirect measure of involuntary muscle contraction to the startle pulse. The mean observed and predicted SIRM data for each genotype for both the prepulse and no prepulse trials are shown in Figure 1. In depth analysis was performed only on the startle-only condition for sensorimotor responses, due to the most important aspects of the prepulse responses being included in measures of sensorimotor gating.

The characterisation of SIRM curve parameters are shown in Figure 2 and detailed statistics in supplementary Table I. R_{max} , which is the theoretical maximum startle response (the upper asymptote) of each individual rat was calculated as shown in Figure 2A. ANCOVAs revealed that both ephrin-A2 genotype ($p < 0.01$) and ephrin-A5 ($p < 0.05$) genotypes exhibited significant differences between the wild-type and knock-out but the interaction wasn't significant ($p > 0.05$). Knocking out the ephrin-A2 gene increased maximum startle magnitude, but knocking out the ephrin-A5 gene decreased maximum startle magnitude, indicating opposite changes in the motor capacity of the ASR pathway.

There were no significant effects of genotype in the y-intercept of the predicted response (i.e. baseline activity levels) (Figure 2B). ES_{50} , the theoretical startle stimulus intensity to produce half-maximum response and a measure of stimulus potency at eliciting a response (Figure 2C), was lowered in ephrin-A2 KO ($p < 0.05$), indicating a shift to the left of the SIRM curve. Ephrin-A5 KO showed no change in ES_{50} ($p > 0.05$). There were no significant effects for ephrin-A2 genotype, or interactions between the genotypes ($p > 0.05$). The Hill slope is a measure of maximum velocity of the SIRM function (Figure 2D). A log transform was applied to Hill slope, due to non-Gaussian distribution of log scales such as decibels, as is also standard in dose-response curve fits, where dose is also expressed as logs. There were no significant effects nor interactions of the genotypes on the log-transformed Hill slope ($p > 0.05$), indicating that all genotypes had similar rates of startle increase over their dynamic response range. Startle threshold (Figure 2E) was increased by ephrin-A5 KO ($p < 0.05$), but no other significant differences due to genotype or interactions were observed ($p > 0.05$).

Startle Magnitude Sensorimotor Gating

Measures of sensorimotor gating were determined by comparing the percent difference (R_{MAX}) or simple differences in SIRM parameters between the startling stimulus alone and prepulse condition. PPI of R_{MAX} , as calculated from Eq. (3) showed no significant differences due to genotype (Figure 3A, all $p > 0.50$), indicating that although there were differences in absolute magnitudes of startle responses (Figure 2A), the inhibition of these responses was proportional and intact in all animals compared to wild-type. The measure of PPI of sensorimotor gating (the slope of the SIRM function, $\Delta \log_{10} \text{Hill slope}$) showed no significant differences due to genotype (Figure 3C, all $p > 0.05$). There were no significant differences in ΔES_{50}

(Figure 3B, $p>0.05$) the stimulus needed for half-maximal response, or with the gating of threshold, Δ Threshold (Figure 3D, $p>0.05$).

Latency of the Acoustic Startle Reflex

Another major component of the ASR assessed was the latency of startle responses. This measure provides information about different components of the startle circuitry. All latency results (Figure 4) were analysed using ANCOVAs from 100-120 dB responses, an area within the upper asymptote of the stimulus intensity-latency curve, to avoid including trials with no startle responses. Stimulus intensity was included as a within-subjects covariate for analysis, yielding single latency mean estimates from this portion of the curve. Detailed statistics on latency results are shown in supplementary Table II.

Initial analysis was conducted on the latency to startle peak (latency to peak) as a measure of maximal startle response output (data not shown). Statistical analysis revealed that the lack of ephrin-A2 resulted in reduced latency to peak ($p<0.01$) whilst for ephrin-A5 it increased latency to peak ($p<0.05$). In order to clarify the source of these latency shifts we separated the responses into different latency components: an estimate of the latency to initiate startle response and estimate of central conduction delay (latency to startle, Figure 4A, C & E), and an estimate of the pure motor component of response latency (motor response latency, Figure 4B, D, & F). Motor response latency was a derived parameter, calculated by taking the latency to startle from latency to startle peak.

The latency to startle response showed differences between groups for the startling stimulus alone condition (Figure 4B). There were significant effects of ephrin-A2 genotype ($p<0.0001$), ephrin-A5 genotype ($p<0.05$), and a significant interaction ($p<0.05$). The effect of ephrin-A2 knock-out was to reduce latency of responses, where ephrin-A5 knock-out showed an increased latency of startle response. Post-hoc analysis using Holm-Bonferroni

corrected t-tests revealed that knocking out the Ephrin-A5 gene only increased latencies in the presence of the wild-type Ephrin-A2 gene (see Figure 4B). Ephrin-A5 knock-out mice also showed reduced gating of latency to startle (Figure 4C, $p < 0.01$), though no other effects or interactions were found ($p > 0.10$).

The motor response latency analysis for the startling stimulus alone condition (Figure 4E, supplementary Table II) revealed that knocking out the ephrin-A2 gene caused a significant reduction in motor response latencies ($p < 0.01$) and conversely, ephrin-A5 knock-out mice had increased motor response latencies ($p < 0.05$). Ephrin-A5 also resulted in reduced gating of motor response latencies (Figure 4F, $p < 0.01$), but there was no significant difference due to ephrin-A2 genotype ($p > 0.10$). There were no interactions for either startling stimulus alone or gating of motor response latency ($p > 0.10$).

The above latency results do not take into account potential differences in startle response probabilities across genotypes (i.e., trials in which no startle response occurred), which may have lead to different latency estimates. We repeated the analyses rejecting all startle response trials that failed to exhibit a startle response at least 3 standard deviations above baseline. This did not change the results (data not shown).

Dopaminergic Cells in the VTA and SNpc

The nucleus area and counts of TH+ cells in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNpc) of each genotype were performed as shown in Figure 5, and analysed using 2-way ANOVAs as shown in supplementary Table III. In the VTA (Figure 5A - C) ephrin-A5 KO increased dopamine cell count ($p < 0.001$) and the density of dopamine neurons ($p < 0.001$) and increased VTA area ($p < 0.01$). There were no main effects of ephrin-A2 genotype, or any interactions in the VTA ($p > 0.05$). In the SNpc (Figure 5D - F) ephrin-A5

had similar effects as those in the VTA. Ephrin-A5 KO resulted in increased TH⁺ cell counts ($p < 0.001$), increased area ($p < 0.01$), and a trend for increased density ($p = 0.051$). There were no differences due to ephrin-A2 genotype (all $p > 0.05$). There were no interactions between genotypes for any condition.

Discussion

Analysis of the ASR and PPI in ephrin-A2 and/or ephrin-A5 knock-out mice revealed that both genes play distinct roles in mediating ASR circuits, but are unlikely to play a role in PPI. Knock-out of either gene resulted in robust changes in startle response magnitude and two different measures of startle latency. However, ephrin-A2 and ephrin-A5 regulate aspects of the ASR differently: ephrin-A2 KO mice have increased startle amplitudes and reduced startle latencies, whilst ephrin-A5 KO mice show opposite effects. In general, the effects of the knock-outs appear to be additive rather than interacting, with ephrin-A2A5^{-/-} mice having phenotypes intermediate between ephrin-A2^{-/-} and ephrin-A5^{-/-}, with startle latency being one exception, where the effect of knocking out the ephrin-A5 gene is only observed if the ephrin-A2 gene is intact. By contrast, changes in dopaminergic cells in the VTA and SNpc were observed only in ephrin-A5 KO mice suggesting that the effects of knocking out the ephrin-A2 gene on startle are not mediated by changes in numbers of dopaminergic neurons in these regions.

The Role of Ephrin-A2 and Ephrin-A5 in the Afferent Startle Pathway

Previous research indicates that ephrin-A2 and ephrin-A5 have anatomical and physiological influences on neurons that underlie afferent portions of the ASR [29-35]. This is behaviourally supported by our data: the reduction in stimulus potency (ES₅₀) for ephrin-A2 KO mice and increased startle thresholds in ephrin-A5 KO mice supports previous evidence that these

proteins regulate auditory brainstem response (ABR) activity [30, 35]. Changes in ephrin-A2 KO ES₅₀ may be due to changes in auditory nerve or cochlear activity; ephrin-A2 is expressed in the cochlea and auditory nerve in adulthood and development [29, 31-34], and KO increases auditory nerve activity [35]. The threshold data in ephrin-A5 KO mice can likely be explained anatomically by the loss of EphA4:Ephrin-A5 interactions in the cochlea resulting in decreased afferent projections to inner hair cells, increased ectopic projections of afferent neurons in the cochlea [30], eventuating in reduced auditory nerve activity [35]. Our study confirms previous evidence of auditory abnormalities in ephrin-A2 and ephrin-A5 KO mice.

Ephrin-A2 and Ephrin-A5 in the Neuromotor Startle Pathway

Changes in other aspects of SIRM curves are less likely to be attributed to changes in the afferent portion of the ASR and suggest changes in neuromotor functioning. Unlike ES₅₀ and startle threshold, R_{MAX} represents a measure of startle reactivity that is relatively independent of auditory sensitivity. R_{MAX} as the asymptote of the SIRM curve represents a prediction of maximum motor output under specific experimental conditions. This independence from auditory sensitivity can be seen by comparing auditory brainstem threshold with R_{MAX} values. For example ephrin-A2A5^{-/-} mice show lower auditory brainstem response (ABR) thresholds compared to ephrin-A2^{-/-} [35], yet exhibit smaller R_{MAX} values than ephrin-A2^{-/-} mice. Furthermore our choice of stimuli also minimises the potential contribution of effects of threshold: we used broadband noise stimuli, whilst changes in ephrin-A2 and ephrin-A5 KO hearing thresholds appear to be frequency-specific [35]. Thus, whilst we cannot completely exclude a contribution of auditory sensitivity to changes in R_{MAX}, it is unlikely that it is a major factor in the effects we have observed on R_{MAX}.

Our observations of altered startle response latencies also supports a role of ephrin-A2 and ephrin-A5 neuromotor development. For both strains, startle latency results contrast with ABR latency data showing no difference or an increase in auditory peripheral latency in ephrin-A2^{-/-} mice [35], and only slightly prolonged (~0.2 – 0.3 ms) latencies in ephrin-A5^{-/-} mice. Therefore changes in auditory processing alone cannot account for increased startle latency observed in this study (in some cases >15 ms), especially in ephrin-A5 KO mice. Rather, differences in startle latencies and startle reactivity in our mice suggest that both ephrin-A2 and ephrin-A5 play important roles in regulating neuromotor development in the ASR. Although indirect, the present study is to our knowledge the first *in vivo* evidence of ephrin-A2 KO enhancing muscle responses. Ephrin-A2 KO mice have an increased R_{MAX}, earlier startle response, earlier muscle contraction peak, and more rapid muscle response. These results all suggest that ephrin-A2 KO increased activity or efficiency in the neuromotor portion of the ASR. Ephrin-A5 KO mice showed increased latency of startle response for all measures of startle, and a reduction in R_{MAX}, suggesting decreased neuromotor performance.

Previous anatomical and physiological data also supports our findings that ephrin-A2 and ephrin-A5 have important roles in neuromotor development. Ephrin-A2 and ephrin-A5 are expressed in the motor neurons and muscle cells from embryonic stages through to adulthood [10, 46-48]. In addition, ephrin-A2 appears to have an important role in guiding motor axons to their correct target in development. Ephrin-A2 is normally expressed in all neurons and axons in the lateral motor column, which under normal conditions project to the dorsal and ventral limb muscles [8, 49], the main muscles that mediate the motor component of the ASR. Ephrin-A2 may also promote neuromotor synaptic stability, it is almost exclusively localised to the neuromuscular junction (NMJ) of myotubes in adulthood [48]. Ephrin-A2 may also affect neuromotor development by exerting an inhibitory effect on muscle precursor cell

1 migration [47]. Thus, it would appear that ephrin-A2 is well-placed to play an important role
 2 in the regulation of multiple parts of the efferent portion of the ASR. In depth *in vivo* physio-
 3 logical characterisation of ephrin-A2^{-/-} neuromuscular development has not been performed.
 4 However there is physiological characterization of the role of ephrin-A5 in the literature [46,
 5 50].
 6
 7
 8
 9

10 The removal of ephrin-A5 may reduce neuromotor performance by changes in NMJ function
 11 and abnormalities in axonal projections. Ephrin-A5 is normally expressed in the NMJ
 12 [50] and loss or overexpression of the protein leads to abnormal neuromuscular innervation
 13 and function (Gluteus and acromiotrapezius muscles [46]; diaphragm [50]). Functionally this
 14 likely results in reduced grip strength in ephrin-A5^{-/-} mice [41], and matches our observations
 15 of reduced R_{MAX} and increased startle latency. Importantly, there appears to be no extra det-
 16 riment in neuromuscular mapping in ephrin-A2A5^{-/-} compared to ephrin-A5^{-/-} [46], suggest-
 17 ing at the very least KO of ephrin-A2 does not exert detrimental effects beyond the effects of
 18 ephrin-A5 KO, if at all. Other findings support the enhancing effects of ephrin-A2 KO; some
 19 features such as the size of the motor pool that are abnormal in ephrin-A5^{-/-}, are normal in
 20 ephrin-A2A5^{-/-} [46], suggesting opposing effects of ephrin-A2 and ephrin-A5. Thus, although
 21 it remains unclear how ephrin-A2 KO affects neuromuscular responses, it seems likely that
 22 ephrin-A5 KO decreased motor responsiveness in our study is a combination of altered NMJ
 23 and abnormal muscle development.
 24
 25
 26
 27
 28
 29
 30
 31
 32
 33
 34
 35
 36
 37
 38
 39
 40
 41
 42
 43
 44
 45
 46
 47
 48
 49
 50

51 *Startle Response Latencies*

52 Prepulse effects on startle latencies are generally complex. Prepulses have been found to pro-
 53 duce no effects (in humans, [51]), increase (in rats [52, 53], in humans [54]) and decrease (in
 54 rats [55-58], in humans [59]) startle latencies. Thus, it is not clear what latency effects of pre-
 55
 56
 57
 58
 59
 60
 61
 62
 63
 64
 65

pulses may relate to. We have found no papers that report on the effects of prepulses on startle latencies on mice. In our hands, wildtype mice and ephrin-A2 KO mice, but not ephrin-A5 KO mice, showed increased startle latencies after presentation of prepulses. However, no effects of knocking out the ephrin-A5 gene were observed on PPI of the startle magnitude, either on R_{MAX} or ES_{50} . Thus, it appears that the effect of prepulses on increasing startle latency in these mice depends on having an intact ephrin-A5 gene.

Our data suggest that there is a central conduction delay, as measured by latency to startle, of ~5 ms, and the delay due to motor components of ~15 ms in ephrin-A5^{-/-} mice. The locus of sensorimotor integration in PPI or ASR is generally considered to be the nucleus reticular pontis caudalis [37]. However, in our ephrin-A5 KO mice the locus of the latency gating deficit appears to be both central and neuromotor. However, the bulk of the delay in startle response and apparent sensorimotor gating deficit appears to be attributable to deficits in muscle response rather than a central deficit contributing to onset latency.

Ephrin-A2 and Ephrin-A5 in PPI and dopamine

Our study demonstrates that ephrin-A2 does not mediate changes in dopamine nuclei area, cell number or density, whilst ephrin-A5 does. From our data it is clear that absence of ephrin-A5 increases TH⁺ cell population number and density. These findings are perhaps not surprising. Previous studies have shown that EphA/Ephrin-A interactions play a role in the development of SNpc projections to the striatum, SNpc volume, and the locomotor stimulant effects of cocaine and amphetamine that increase extracellular dopamine levels [60]. Furthermore, ephrin-A5 is expressed in the nucleus accumbens (ventral striatum), a target of midbrain dopamine axons in the VTA and SNpc and regulator of PPI [37, 61]. However our immunohistochemical data appears to be inconsistent with other findings, which found the total number of midbrain dopaminergic neurons was not changed in ephrin-A5^{-/-} mice [7]. In

support of our results, our TH⁺ cell counts in WT and ephrin-A2^{-/-} mice are similar to previous reports in C57BL/6 mice [62].

From our results alone it is difficult to postulate the precise role that changes in the VTA and SNpc may play in the observed changes in the ASR. Two of the key findings in our study were that ephrin-A5 KO results in reduced startle magnitude, and longer startle latencies. On the surface it would be easy to postulate that an increase in TH⁺ cell number or density in ephrin-A5^{-/-} mice may result in increased ascending dopamine projections and dopamine activity, resulting in prolonged latencies [63], and reduced startle amplitudes [12, 16]. However, ephrin-A5^{-/-} mice show reduced ascending dopamine connections [5, 7], reduced dopamine-related protein expression, increased dopamine turnover, and reduced dopamine content in the striatum [41]. Furthermore, we did not demonstrate any deficits in sensorimotor gating that are normally associated with hyperdopaminergia [12, 15, 16]. In addition the possibility of low dopamine levels would also not explain the startle magnitude or latency effects of ephrin-A2 KO since dopamine antagonists do not invoke changes in startle magnitude [15], and high dopamine levels would likely result in decreases in startle amplitude in mice rather than the increases observed [12, 16]. Without further anatomical or pharmacological data, such as administration of dopamine receptor agonist and antagonists, a role of dopamine cannot be ruled out in ephrin-A5 KO mice, but as with ephrin-A2 KO, our data suggest that it is not likely to be the main mediator of changes observed in startle size or latency.

Conclusion

Our data suggest that both ephrin-A2 and ephrin-A5 play roles in the ASR, but do not alter measures of sensorimotor gating such as PPI. Ephrin-A5 also appears to be involved in the development of dopaminergic cells in the VTA and SNpc. Previous literature hints at possible ways that ephrin-A2 and ephrin-A5 may mediate their effects on ASR. Some effects may be

1 due to previously demonstrated changes in auditory sensitivity or dopamine turnover. The
2 majority of the changes however, appear to be mediated by the effects of ephrin-A2 and eph-
3 rin-A5 KO on the interaction of motor neurons and their muscle targets.
4
5
6
7
8

9 *Acknowledgements*

10 We would like to thank Clarissa Yates for her invaluable assistance in performing experi-
11 ments. We would also like to acknowledge the contributions of Donald Robertson for com-
12 ments on drafts of the manuscript. Funded by an Australian Postgraduate Award to NJY, a
13 National Health and Medical Research Senior Research Fellowship to JR, and by the Univer-
14 sity of Western Australia to MTMI.
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Figure Captions:

Figure 1. Stimulus intensity-response magnitude (SIRM) curves. The symbols shown illustrate the mean response at each stimulus intensity $\pm$ SEM. The curves are the predicted group SIRM means based upon the fitting to Eq. (1) and correcting for age and mass effects.

Figure 2. Parameters derived from fitting startle pulse only responses to sigmoid curves as in Eq. 1. A), the maximum predicted response, R_{MAX} . B), y-intercept of the predicted response, y_0 . C), theoretical stimulus intensity to reach half-maximum response, ES_{50} . D) The $\log_{10}$ of the Hillslope, a measure of maximum curve velocity at zero acceleration. E), estimated threshold for startle. Bars represent means adjusted for age and weight effects $\pm$ SEM, using ANCOVA models with ephrin-A2 and ephrin-A5 genotypes as main factors. Significant differences: * $p < 0.05$, ** $p < 0.01$.

Figure 3. Prepulse inhibition of curve parameters derived from fitting startle response to Eq. 1. A), prepulse inhibition of R_{MAX} . B), the shift in theoretical stimulus intensity to reach half-maximum response ES_{50} . C), the difference in $\log_{10}(\text{Hillslope})$, a measure of maximum curve gradient. D), estimated change in threshold for startle. Bars represent means adjusted for age and weight effects $\pm$ SEM using ANCOVA models with ephrin-A2 and ephrin-A5 genotypes as main factors.

Figure 4. Measures of startle response latency. A) to C) show latency to startle analyses, whilst D) to F) motor response latencies. Raw stimulus - intensity response latency curves are shown in A) and D). ANCOVA adjusted mean latencies over 100-120 dB in the startling stimulus alone condition are shown in B) and E). Mean gating of startle latency for stimulus intensities of 100-120 dB shown in C) and F). Statistics are calculated from mixed-model

1 ANCOVAs, ephrin-A2 and ephrin-A5 genotypes as main factors, within-subject covariate of
2 stimulus intensity, between-subject covariates of age and mass. Brackets in B) indicate sig-
3 nificant differences derived from Holm-Bonferroni corrected t-tests between individual geno-
4 types. Analysis was chosen over 100-120 dB due to decreased probabilities of response with
5 lower stimulus intensities. Significant differences: * $p<0.05$, ** $p<0.01$, *** $p<0.001$,
6 **** $p<0.0001$.

19 [COLOUR ONLINE ONLY]

22 Figure 5. TH+ cells in the VTA (A to C) and SNpc (D to E). Total TH+ cell counts in (A &
23 D), size of TH+ nucleus area (B & E), and density of TH+ cells (C & F) in the VTA and
24 SNpc. Effects reported are results from a 2-way ANOVAs for ephrin-A2 and ephrin-A5
25 genotypes. Bars represent mean $\pm$ SEM, significant differences: * $p<0.05$, ** $p<0.01$, ***
26 $p<0.001$. Example tissue staining is shown for wild-type (H) and ephrin-A2A5^{-/-} (I) at a simi-
27 lar level of the midbrain containing parts of the VTA and SNpc. Scale bars = 1 mm.

References

- [1] Wilks TA, Rodger J, Harvey AR. A role for ephrin-As in maintaining topographic organization in register across interconnected central visual pathways. *European Journal of Neuroscience*. 2010;31:613-22.
- [2] Haustead DJ, Lukehurst SS, Clutton GT, Bartlett CA, Dunlop SA, Arrese CA, et al. Functional Topography and Integration of the Contralateral and Ipsilateral Retinocollicular Projections of Ephrin-A-/- Mice. *The Journal of Neuroscience*. 2008;28:7376-86.
- [3] Depaepe V, Suarez-Gonzalez N, Dufour A, Passante L, Gorski JA, Jones KR, et al. Ephrin signalling controls brain size by regulating apoptosis of neural progenitors. *Nature*. 2005;435:1244-50.
- [4] Klein R. Eph/ephrin signaling in morphogenesis, neural development and plasticity. *Current Opinion in Cell Biology*. 2004;16:580-9.
- [5] Deschamps C, Faideau M, Jaber M, Gaillard A, Prestoz L. Expression of ephrinA5 during development and potential involvement in the guidance of the mesostriatal pathway. *Experimental Neurology*. 2009;219:466-80.
- [6] Lein ES, Hawrylycz MJ, Ao N, Ayres M, Bensinger A, Bernard A, et al. Genome-wide atlas of gene expression in the adult mouse brain. *Nature*. 2007;445:168-76.
- [7] Cooper MA, Kobayashi K, Zhou R. Ephrin-A5 regulates the formation of the ascending midbrain dopaminergic pathways. *Developmental Neurobiology*. 2009;69:36-46.
- [8] Eberhart J, Barr J, O'Connell S, Flagg A, Swartz ME, Cramer KS, et al. Ephrin-A5 Exerts Positive or Inhibitory Effects on Distinct Subsets of EphA4-Positive Motor Neurons. *The Journal of Neuroscience*. 2004;24:1070-8.
- [9] Wang H, Chadaram SR, Norton AS, Laskowski MB. Development of inhibition by ephrin-A5 on outgrowth of embryonic spinal motor neurites. *Journal of Neurobiology*. 2001;47:233-43.
- [10] Eberhart J, Swartz M, Koblar SA, Pasquale EB, Tanaka H, Krull CE. Expression of EphA4, Ephrin-A2 and Ephrin-A5 during Axon Outgrowth to the Hindlimb Indicates Potential Roles in Pathfinding. *Developmental Neuroscience*. 2000;22:237-50.
- [11] Swartz ME, Eberhart J, Pasquale EB, Krull CE. EphA4/ephrin-A5 interactions in muscle precursor cell migration in the avian forelimb. *Development*. 2001;128:4669-80.
- [12] Ralph RJ, Varty GB, Kelly MA, Wang Y-M, Caron MG, Rubinstein M, et al. The Dopamine D2, but not D3 or D4, Receptor Subtype is Essential for the Disruption of Prepulse Inhibition Produced by Amphetamine in Mice. *J Neurosci*. 1999;19:4627-33.
- [13] Martin-Iverson M, Else D. PHNO, a selective dopamine D2 receptor agonist, does not reduce prepulse inhibition of the startle reflex in rats. *Psychopharmacologia*. 2000;151:38-48.
- [14] Martin-Iverson M, Stevenson K. Apomorphine effects on emotional modulation of the startle reflex in rats. *Psychopharmacology*. 2005;181:60-70.
- [15] Ralph-Williams RJ, Lehmann-Masten V, Geyer MA. Dopamine D1 rather than D2 receptor agonists disrupt prepulse inhibition of startle in mice. *Neuropsychopharmacology*. 2003;28:108-18.
- [16] Ralph-Williams RJ, Lehmann-Masten V, Otero-Corchon V, Low MJ, Geyer MA. Differential Effects of Direct and Indirect Dopamine Agonists on Prepulse Inhibition: A Study in D1 and D2 Receptor Knock-Out Mice. *The Journal of Neuroscience*. 2002;22:9604-11.
- [17] Fleshler M. Adequate acoustic stimulus for startle reaction in the cat. *Journal of comparative and physiological psychology*. 1965;60:200.
- [18] Cook EW, Hawk LW, Davis TL, Stevenson VE. Affective individual differences and startle reflex modulation. *Journal of Abnormal Psychology*. 1991;100:5.
- [19] Davis M, Gendelman D, Tischler M, Gendelman P. A primary acoustic startle circuit: lesion and stimulation studies. *The Journal of Neuroscience*. 1982;2:791-805.

- [20] Willott JF, Carlson S, Chen H. Prepulse inhibition of the startle response in mice: Relationship to hearing loss and auditory system plasticity. *Behavioral neuroscience*. 1994;108:703-13.
- [21] Jaspers RMA, Muijser H, Lammers JHCM, Kulig BM. Mid-frequency hearing loss and reduction of acoustic startle responding in rats following trichloroethylene exposure. *Neurotoxicology and Teratology*. 1993;15:407-12.
- [22] Carlson S, Willott JF. The behavioral salience of tones as indicated by prepulse inhibition of the startle response: relationship to hearing loss and central neural plasticity in C57BL/6J mice. *Hearing Research*. 1996;99:168-75.
- [23] Parham K, Willott JF. Acoustic startle response in young and aging C57BL/6J and CBA/J mice. *Behavioral neuroscience*. 1988;102:881.
- [24] Paylor R, Crawley JN. Inbred strain differences in prepulse inhibition of the mouse startle response. *Psychopharmacology*. 1997;132:169-80.
- [25] Hince DA, Martin-Iverson MT. Differences in prepulse inhibition (PPI) between Wistar and Sprague-Dawley rats clarified by a new method of PPI standardization. *Behavioral neuroscience*. 2005;119:66.
- [26] Groves PM, Thompson RF. Habituation: a dual-process theory. *Psychological Review*. 1970;77:419.
- [27] Scholes KE, Martin-Iverson MT. Disturbed prepulse inhibition in patients with schizophrenia is consequential to dysfunction of selective attention. *Psychophysiology*. 2010;47:223-35.
- [28] Vrana SR, Spence EL, Lang PJ. The startle probe response: A new measure of emotion? *Journal of Abnormal Psychology*. 1988;97:487.
- [29] Pickles JO, Claxton C, Van Heumen WRA. Complementary and layered expression of Ephs and ephrins in developing mouse inner ear. *The Journal of Comparative Neurology*. 2002;449:207-16.
- [30] Defourny J, Poirrier A-L, Lallemand F, Mateo Sánchez S, Neef J, Vanderhaeghen P, et al. Ephrin-A5/EphA4 signalling controls specific afferent targeting to cochlear hair cells. *Nat Commun*. 2013;4:1438.
- [31] Bianchi LM, Gale NW. Distribution of Eph-related molecules in the developing and mature cochlea. *Hearing Research*. 1998;117:161-72.
- [32] Bianchi LM, Liu H. Comparison of Ephrin-A ligand and EphA receptor distribution in the developing inner ear. *The Anatomical Record*. 1999;254:127-34.
- [33] Siddiqui SA, Cramer KS. Differential expression of Eph receptors and ephrins in the cochlear ganglion and eighth cranial nerve of the chick embryo. *The Journal of Comparative Neurology*. 2005;482:309-19.
- [34] Lee KH, Warchol ME. Ephrin A2 May Play a Role in Axon Guidance during Hair Cell Regeneration. *The Laryngoscope*. 2005;115:1021-5.
- [35] Yates N, Robertson D, Martin-Iverson M, Rodger J. Auditory Brainstem Responses of Ephrin-A2-/-, Ephrin-A5-/-and Ephrin-A2A5-/-Mice. *Audiology and Neurotology*. 2014;19:115-26.
- [36] Swerdlow NR, Caine SB, Braff DL, Geyer MA. The neural substrates of sensorimotor gating of the startle reflex: a review of recent findings and their implications. *Journal of Psychopharmacology*. 1992;6:176-90.
- [37] Swerdlow N, Geyer M, Braff D. Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. *Psychopharmacology*. 2001;156:194-215.
- [38] Beckstead RM, Domesick VB, Nauta WJH. Efferent connections of the substantia nigra and ventral tegmental area in the rat. *Brain Research*. 1979;175:191-217.
- [39] Allen Institute for Brain Science. Allen Mouse Brain Atlas [Internet]. Available from: <http://mouse.brain-map.org/>. 2014.
- [40] Wang L, Marquardt T. What axons tell each other: axon-axon signaling in nerve and circuit assembly. *Current Opinion in Neurobiology*. 2013;23:974-82.
- [41] Sheleg M, Yochum CL, Wagner GC, Zhou R, Richardson JR. Ephrin-A5 deficiency alters sensorimotor and monoaminergic development. *Behavioural Brain Research*. 2013;236:139-47.

- [42] Hunter KP, Willott JF. Aging and the auditory brainstem response in mice with severe or minimal presbycusis. *Hearing Research*. 1987;30:207-18.
- [43] Zheng QY, Johnson KR, Erway LC. Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. *Hearing Research*. 1999;130:94-107.
- [44] Stoddart C, Noonan J, Martin-Iverson M. Stimulus quality affects expression of the acoustic startle response and prepulse inhibition in mice. *Behavioral neuroscience*. 2008;122:516.
- [45] Fu Y, Yuan Y, Halliday G, Rusznák Z, Watson C, Paxinos G. A cytoarchitectonic and chemoarchitectonic analysis of the dopamine cell groups in the substantia nigra, ventral tegmental area, and retrorubral field in the mouse. *Brain Structure and Function*. 2012;217:591-612.
- [46] Feng G, Laskowski MB, Feldheim DA, Wang H, Lewis R, Frisen J, et al. Roles for Ephrins in Positionally Selective Synaptogenesis between Motor Neurons and Muscle Fibers. *Neuron*. 2000;25:295-306.
- [47] Stark DA, Karvas RM, Siegel AL, Cornelison DDW. Eph/ephrin interactions modulate muscle satellite cell motility and patterning. *Development*. 2011;138:5279-89.
- [48] Lai K-O, Ip FCF, Cheung J, Fu AKY, Ip NY. Expression of Eph Receptors in Skeletal Muscle and Their Localization at the Neuromuscular Junction. *Molecular and Cellular Neuroscience*. 2001;17:1034-47.
- [49] Eberhart J, Swartz ME, Koblar SA, Pasquale EB, Krull CE. EphA4 Constitutes a Population-Specific Guidance Cue for Motor Neurons. *Developmental Biology*. 2002;247:89-101.
- [50] Yumoto N, Wakatsuki S, Kurisaki T, Hara Y, Osumi N, Frisén J, et al. Meltrin β /ADAM19 Interacting with EphA4 in Developing Neural Cells Participates in Formation of the Neuromuscular Junction. *PLoS ONE*. 2008;3:e3322.
- [51] Blumenthal TD. Inhibition of the human startle response is affected by both prepulse intensity and eliciting stimulus intensity. *Biological Psychology*. 1996;44:85-104.
- [52] Seaman RL, Beblo DA, Raslear TG. Modification of acoustic and tactile startle by single microwave pulses. *Physiology & Behavior*. 1994;55:587-95.
- [53] Fitting S, Booze RM, Mactutus CF. Neonatal hippocampal Tat injections: developmental effects on prepulse inhibition (PPI) of the auditory startle response. *International Journal of Developmental Neuroscience*. 2006;24:275-83.
- [54] Abel K, Waikar M, Pedro B, Hemsley D, Geyer M. Repeated testing of prepulse inhibition and habituation of the startle reflex: a study in healthy human controls. *Journal of Psychopharmacology*. 1998;12:330-7.
- [55] Kodsi MH, Swerdlow NR. Ventral pallidal GABA-A receptors regulate prepulse inhibition of acoustic startle. *Brain Research*. 1995;684:26-35.
- [56] Fitting S, Booze RM, Hasselrot U, Mactutus CF. Intrahippocampal injections of Tat: Effects on prepulse inhibition of the auditory startle response in adult male rats. *Pharmacology Biochemistry and Behavior*. 2006;84:189-96.
- [57] Fitting S, Booze RM, Mactutus CF. Neonatal Intrahippocampal Glycoprotein 120 Injection: The Role of Dopaminergic Alterations in Prepulse Inhibition in Adult Rats. *Journal of Pharmacology and Experimental Therapeutics*. 2006;318:1352-8.
- [58] Fitting S, Booze RM, Mactutus CF. Neonatal intrahippocampal gp120 injection: An examination early in development. *NeuroToxicology*. 2007;28:101-7.
- [59] Kedzior KK, Koch M, Basar-Eroglu C. Prepulse inhibition (PPI) of auditory startle reflex is associated with PPI of auditory-evoked theta oscillations in healthy humans. *Neuroscience Letters*. 2006;400:246-51.
- [60] Sieber B-A, Kuzmin A, Canals JM, Danielsson A, Paratcha G, Arenas E, et al. Disruption of EphA/ephrin-A signaling in the nigrostriatal system reduces dopaminergic innervation and dissociates behavioral responses to amphetamine and cocaine. *Molecular and Cellular Neuroscience*. 2004;26:418-28.

[61] Swanson LW. The projections of the ventral tegmental area and adjacent regions: A combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain Research Bulletin*. 1982;9:321-53.

[62] Nelson EL, Liang CL, Sinton CM, German DC. Midbrain dopaminergic neurons in the mouse: Computer-assisted mapping. *The Journal of Comparative Neurology*. 1996;369:361-71.

[63] Naudin B, Canu S, Costentin J. Effects of various direct or indirect dopamine agonists on the latency of the acoustic startle response in rats. *J Neural Transmission*. 1990;82:43-53.