



Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr



Research report

Abnormal strategies during visual discrimination reversal learning in *ephrin-A2*^{-/-} mice

S. Arnall^{a,1}, L.Y. Cheam^{a,1}, C. Smart^{a,1}, A. Rengel^a, M. Fitzgerald^a, J.P. Thivierge^b, J. Rodger^{a,*}

^a Experimental and Regenerative Neurosciences, School of Animal Biology, University of Western Australia, Crawley, WA 6009, Australia

^b Department of Psychological and Brain Sciences, Indiana University, IN 47405, USA

ARTICLE INFO

Article history:

Received 15 December 2009
Received in revised form 14 January 2010
Accepted 18 January 2010
Available online xxx

Keywords:

Topography
Perseveration
Strategy
Bias
Prefrontal cortex
Orbitofrontal cortex
Nucleus accumbens
Thalamus
Ephrin-A knockout

ABSTRACT

Eph receptors and ephrins are involved in establishing topographic connectivity in primary sensory brain regions, but also in higher order structures including the cortex and hippocampus. *Ephrin-A2*^{-/-} mice have abnormal topography in the primary visual system but have normal visual and learning performance on a simple visual discrimination task. Here we use signal detection theory to analyse learning behaviour of these mice. Wild-type (WT) and *ephrin-A2*^{-/-} (KO) mice performed equally well in a two-stimulus visual discrimination task, with similar learning rates and response latencies. However, during reversal learning, when the rewarded stimulus was switched, the two genotypes exhibited differences in response strategies: while WT mice favoured a win-stay strategy, KO mice remained relatively neutral. KO mice also exhibited a stronger lateralization bias in the initial stages of learning, choosing the same arm of the maze with high probability. In addition, use of a Bayesian “optimal observer” revealed that compared to WT, KO mice adapted their decisions less rapidly to a change in stimulus-reward relationship. We suggest that the misexpression of ephrin-A2 may lead to abnormal connectivity in regions known for their involvement in reversal learning and perseverative behaviours, including thalamic–prefrontal cortical–striatal circuitry and particularly orbitofrontal cortex. The implication is that topographic organisation of higher order brain regions may play an important role in learning and decision making.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Eph receptors and ephrin ligands are cell-surface proteins that have been shown to contribute to many processes in the developing brain including cell proliferation, brain regional organisation and neuronal connectivity [20]. A key function of these proteins is to guide growing axons to precise targets within brain regions [5,23]. Receptor–ligand binding results in bidirectional activation of intracellular signalling pathways, commonly resulting in cytoskeletal rearrangements that underlie axon guidance [17]. Because the proteins are frequently expressed as gradients in interconnected brain regions [5,23], Ephs and ephrins are strongly associated with the development of topographically organised projections [11,30], particularly in primary sensory regions including the visual, auditory and olfactory systems [6,7,24]. Gradients of Ephs and ephrins have also been detected in topographically organised regions that are thought to support complex processes including learning, mem-

ory and cognition; these regions include the striatum, cortex and hippocampus [9,18,31].

Transgenic and knockout mouse lines have been created to elucidate the molecular functions of Eph/ephrins in various brain regions, and have generally demonstrated abnormal connectivity and/or altered synaptic plasticity. However, relatively few studies have taken advantage of these mice to examine the behavioural consequences of these abnormalities, despite the opportunity to gain insight into the relationship between brain connectivity and behaviour. A significant limitation has been the difficulty in interpreting data from constitutive knockouts. For example, *EphA4*^{-/-} mice have reduced locomotor activity [19], ruling out most behavioural tests. Although most behavioural studies have demonstrated impaired learning and memory when Eph/ephrin signalling is disrupted, they all acknowledge that unreported sensory or motor deficits may confound results. *EphB2*^{-/-} mice display subtle deficits in the Morris Water maze, but were impaired from the very first trial, making results difficult to interpret [13]. Similarly, a recent study in *EphA6*^{-/-} mice identified deficits in learning and memory on spatial and fear conditioning tasks [28], but abnormal baseline freezing behaviour meant that the authors could not distinguish between abnormal sensory processing or a learning deficit. The strongest support for a role for Eph/ephrin signalling in learning and memory comes from studies which infused

* Corresponding author at: Experimental and Regenerative Neurosciences, School of Animal Biology M317, University of Western Australia, Crawley, WA 6009, Australia. Tel.: +61 8 6488 2245; fax: +61 8 6488 7527.

E-mail address: jrodger@cyllene.uwa.edu.au (J. Rodger).

¹ These authors contributed equally to the work.

recombinant EphA or ephrin-A proteins into the hippocampus and demonstrated impaired spatial learning [10]. However this methodology tested the immediate impact of eph/ephrin blockade on learning and did not investigate the consequences of miswiring as a result of abnormal development in a knockout mouse.

One of the best studied knockout strains is the *ephrin-A2*^{-/-} mouse, which was originally created to investigate the role of this protein in guiding retinal ganglion cell axons to their targets in the midbrain, the superior colliculus and lateral geniculate nucleus [7]. Disruption of the *ephrin-A2* gene results in moderate topographic defects in these projections, which persist into adulthood. Retinal ganglion cells form appropriately located terminations within the SC and LGN, but also form aberrant ectopic projections [7], some of which are functional [15]. Nonetheless, knockout mice display normal performance on several visually guided behaviours, including visual placing, pupil reflex and visual acuity and have normal overall activity levels [15]. As a result, any behavioural impairment on a visual discrimination task are likely to be attributed to abnormal processing outside of the primary sensory brain regions. Ephrin-A2 is strongly expressed throughout the cortex, hippocampus and striatum [1,9,18], suggesting that these regions will be abnormally wired in the knockout.

Here we trained mice on a visual discrimination task and used signal detection theory to analyse behaviour during initial learning, and during reversal, when the opposite stimulus was rewarded. We hypothesised that WT and KO mice would show similar rates of learning as suggested by our previous work [15], but might employ different strategies to acquire the initial or reversed task. In support of this hypothesis, we show that wild-type (WT) and *ephrin-A2*^{-/-} (KO) mice performed equally well in a two-stimulus visual discrimination task, with similar learning rates and accuracy. However, during reversal, the two genotypes exhibited differences in response strategies: while WT mice favoured a win-stay strategy, KO mice remained relatively neutral. KO mice also exhibited a stronger lateralization bias in the initial stages of learning, preferring the same (left) arm of the maze. In addition, use of a Bayesian “optimal observer” analysis revealed that compared to WT, KO mice adapted their decisions less rapidly to a change in stimulus–reward relationship, further identifying abnormal strategies during reversal learning. These results suggest abnormalities in prefrontal cortical regions, and/or in thalamic–prefrontal cortical–ventral striatal circuitry that are essential for flexibility in learning and decision making.

2. Methods

2.1. Animals and housing

Age-matched (6–8 weeks) wild-type (WT; C57BL/6J; $n = 5$) and knockout mice (KO; *ephrin A2*^{-/-}; $n = 5$), were obtained from a breeding colony at the University of Western Australia. Knockout mice were a generous gift from Feldheim et al. [7] and were rederived on a C57Bl/6J background. A C57Bl/6J-congenic strain was then produced following standard procedures, by backcrossing the *ephrin-A2*^{-/-} strain onto a C57Bl/6 background for 10 generations [15,29]. Same-sex (female) and same-strain animals were group-housed in standard cages (45 cm × 29 cm × 12 cm) under a 12-h light/dark schedule (lights on 7 a.m. to 7 p.m.) in controlled environmental conditions of 22 ± 2 °C and 50 ± 10% relative humidity. Food (Rat & Mouse Chow, Speciality Foods, Glen Forrest, Western Australia) and water were provided *ad libitum*. Animals were handled daily (2 × 10 min/mouse) for one week prior to experimentation upon which food deprivation commenced (1–2 pellets daily intake) to facilitate rapid learning. Individual weights were monitored to ensure the animals remained healthy. All trials were conducted within the same six hours of the light cycle. The study was approved by the Animal Ethics Committee of the UWA (AEC 03/100/526) and performed in accordance with Principles of Laboratory Care (NIH publication no. 86-23, revised 1985).

2.2. Visual discrimination (match-to-sample) task

Mice were habituated to a Y-shape maze for five consecutive days prior to training. The floor of the box was covered with wood shavings identical to that of the subjects' housing. A laminated card displaying a visual stimulus was placed at the

end of each arm. The visual stimuli consisted of laminated squares of paper (6 cm² each) with either vertical black and white stripes at 0.37 cycles per degree (a spatial frequency that can be distinguished by both WT and KO mice [15]) or a solid 50% grey square of the same luminance as the striped pattern. Correct choices were immediately rewarded (peanut butter), and after each attempt, mice were returned to the starting position by hand for the next trial. Initial trials consisted of ten attempts twice daily which was later increased to fifty attempts daily as competency increased. Pattern locations for each choice were randomly allocated to the arms before each trial, but kept constant for all mice. Individuals were scored on their choice after crossing a line halfway down the arm at which point timing ceased. Subjects were deemed to have made a non-choice if they had not entered any arm after 60 s. Accuracy (% correct choices) and response latency (seconds) were recorded. Criterion performance was set at 75% correct responses for two consecutive days following standard procedures [15,26]. After each trial, wood shavings were mixed in order to disrupt any olfactory information.

2.3. Experimental design

During the first learning phase (Learning Phase 1, defined by the time taken to reach criterion; 12 days), mice were rewarded when the striped card was selected. After criterion was reached, trials continued for 5 days to collect data during performance on the learnt task (Learnt Phase). Mice then underwent a second learning phase (Learning Phase 2, defined by the time taken to reach criterion; 8 days), when they were rewarded when the previously incorrect stimulus (solid grey square) was selected. We verified in a separate cohort of mice that there was no innate preference for striped or grey patterns in either WT or KO mice (data not shown, also in [15]). This was confirmed in the present study by similar (50%) accurate response rates at the beginning of Learning Phases 1 and 2 for both genotypes. For this reason, the starting stimulus was kept constant across groups to facilitate the experimental procedure.

2.4. Analysis

Task performance (days to criterion) and decision time were analysed in STATVIEW (version 5.0.1, 1998) using non-parametric survival tests (Mantel–Cox). Animals that failed to reach criterion were censored from further analysis in the specific time period.

For signal detection theory analysis, responses were scored and categorized based on the response type. A correct switch indicated a response in which the animal correctly recognized a change in location of the target stimulus, while a correct repeat occurred when the animal correctly repeated their response. Incorrect response (switch or repeat) occurred when the animal made an incorrect decision or failed to respond (timed out). Non-choices (i.e. where no response is made after 60 s) were included in the analysis and considered to be a switch to an incorrect strategy (one of non-response). Non-choices were therefore scored as ‘Incorrect Switches’.

Responses were analysed using three measures as follows:

- D Prime (d') is a measure of signal to noise ratio [12] and was calculated as:

$$d' = \frac{z(H) - z(F)}{\sqrt{2}}$$

where $z(H)$ and $z(F)$ represent the z -distribution scores of the number of Correct Repeats and Incorrect Repeats. The formula has been corrected for use with two-alternative forced choice tasks by dividing the z -scores by the square of two. $d' > 0.8$ indicate high sensitivity and $d' < 0$ indicate extreme insensitivity [21].

- Index Y (I_Y) determines whether the animals have an innate preference to either side of the maze [27] and is calculated as:

$$I_Y = \frac{|\text{Left} - \text{Right}|}{\text{Total Correct}}$$

where Left and Right are the number of correct responses to the left and right respectively and Total Correct is the total amount of correct responses. A value of zero represents an absence of bias.

- The strategy index (I_X) examines response bias, and is calculated as:

$$I_X = \frac{P(r/s) - P(s/r) + 1}{2}$$

where $P(r/s)$ is the probability of making an incorrect repeat when a switch is required and $P(s/r)$ is the probability of making an incorrect switch when a repeat is required. $I_X > 0.5$ indicates a tendency towards repeating responses (‘win-repeat’ strategy) while $I_X < 0.5$ indicates a tendency towards switching responses (‘win-switch’ strategy) [27].

Data were analysed using SPSS Statistics (version 17, 2008). A mixed-design ANOVA with Day of Training ($n, n + 1, \dots$) as the repeated measure and Genotype (wild-type or *ephrin-A2*^{-/-}) as the between-subjects measure was used to assess d' ,

I_x and I_y across the three training periods (Learning Phase 1, Learnt Phase, Learning Phase 2).

2.5. Bayesian-optimal observer

We defined a Bayesian “optimal observer” to describe what the best decision should be (from a statistical point of view) on every trial over all days of training.

The optimal utility depends on the scenarios L_i presented at every trial. Two scenarios are possible: (1) the striped pattern is in the left arm of the maze; or (2) the striped pattern is in the right arm of the maze. The optimal utility also depends on the decisions a_i (representing two choices, moving left or moving right). On any given trial t , the optimal decision $a_{opt}(L_i)$ that will yield the highest expected reward r is

$$a_t^{opt}(L_i) = \arg \max_{a_i} p(r|L_i, a_i)_t \tag{1}$$

By applying Bayes' rule, we obtain $p(r|L_i, a_i)_t$ (the probability of a reward r given the joint probability of a scenario L_i and a decision a_i):

$$p(r|L_i, a_i)_t = \frac{p(L_i, a_i|r)_t \cdot p(r)_t}{p(L_i, a_i)_t} \tag{2}$$

Initially, we devised a Bayesian model where all probabilities were set to 0.5. Then, for each subject, we computed Eq. (1). The goal of Eq. (1) is to determine which of the two potential decisions (moving right or moving left) is optimal in terms of reward expectations. In this way, moving right (a_1) is more optimal than moving left (a_2) if $a_1 > a_2$, and moving left is more optimal if $a_1 < a_2$. After an optimal decision is determined on each trial, we updated $p(L_i, a_i|r)_t$, $p(r)_t$, and $p(L_i, a_i)_t$ by taking into account information from all trials that the subject had seen. Taking $p(r)_t$ as an example, this updating was performed as follows:

$$p(r)_t = p(r)_{t-1} + (\eta \cdot (\bar{p}(r)_t - p(r)_{t-1})) \tag{3}$$

where $\bar{p}(r)_t$ is the proportion of a trials (from trial 1, ..., t) that yielded a reward.

The parameter η in Eq. (3) can be adjusted to fit the Bayesian-optimal observer. We explored a total of 10,000 different values of η ranging from 0.00001 to 10 in increments of 0.001. For each value of η , we computed the decision of the ideal observer at every trial of phases I and II. In order to compare these “Bayesian-optimal decisions” to the decisions made by the subjects, we focused on the decisions to the left arm of the maze (because only two choices were possible, focusing on decisions to the right arm would yield the same conclusions). For each trial, we computed the proportion of subjects that made a left arm decision. Then, we used a Pearson correlation to compare this proportion to the Bayesian-optimal decision. The value of η that yielded the highest correlation was deemed to provide the best fit between the Bayesian model and the experimental data. Of course, it is possible that a better fit could be obtained if we considered different values of η for phases I and II independently. For the purposes of the current study, we restricted our analyses to a single value of η that resulted in the highest correlation between the Bayesian model and experimental data for the first 40 trials (entire day of training) at the beginning of phase II. Different values of η were obtained for the two experimental groups (wild-type and KO mice) independently. In this context, a high value of η would suggest that subjects adapted rapidly to a change in the stimulus–reward relationship. That is, a high value of η would suggest that subjects were prone to overwrite distant knowledge in favour of information acquired during recent trials. Conversely, a low value of η would suggest a resistance to overwrite distant knowledge relative to the stimulus–reward relationship.

3. Results

3.1. Task performance

During training phase 1, no difference was detected between genotypes in the number of days to reach criterion (WT: mean (M) = 13 ± 0.86 days; *ephrin-A2*^{-/-}: M = 11 ± 0.41 days; P = 0.797; Fig. 1A). One *ephrin-A2*^{-/-} mouse failed to reach criterion and was removed from further analysis during this phase. All mice maintained criterion performance during the Learnt Phase (Fig. 1A). During Learning Phase 2, no difference in learning rate was detected between genotypes (WT: M = 9 ± 1.08 days; *ephrin-A2*^{-/-}: M = 10 ± 1.3 days; P = 0.732) (Fig. 1A). Time to criterion was slightly less in training phase 2 than phase 1, although this was not significant (P > 0.578). Response latency decreased as animals became proficient with the task, but there was no difference between genotypes at any time (Fig. 1A). The average time taken for mice to make a response decreased from Day 1 (M = 11.31 ± 1.63 s SD) to Day 25 (M = 1.84 ± 0.16 s SD). Training time was greater during training phase 1 compared to phase 2, P < 0.05 (Fig. 1A).

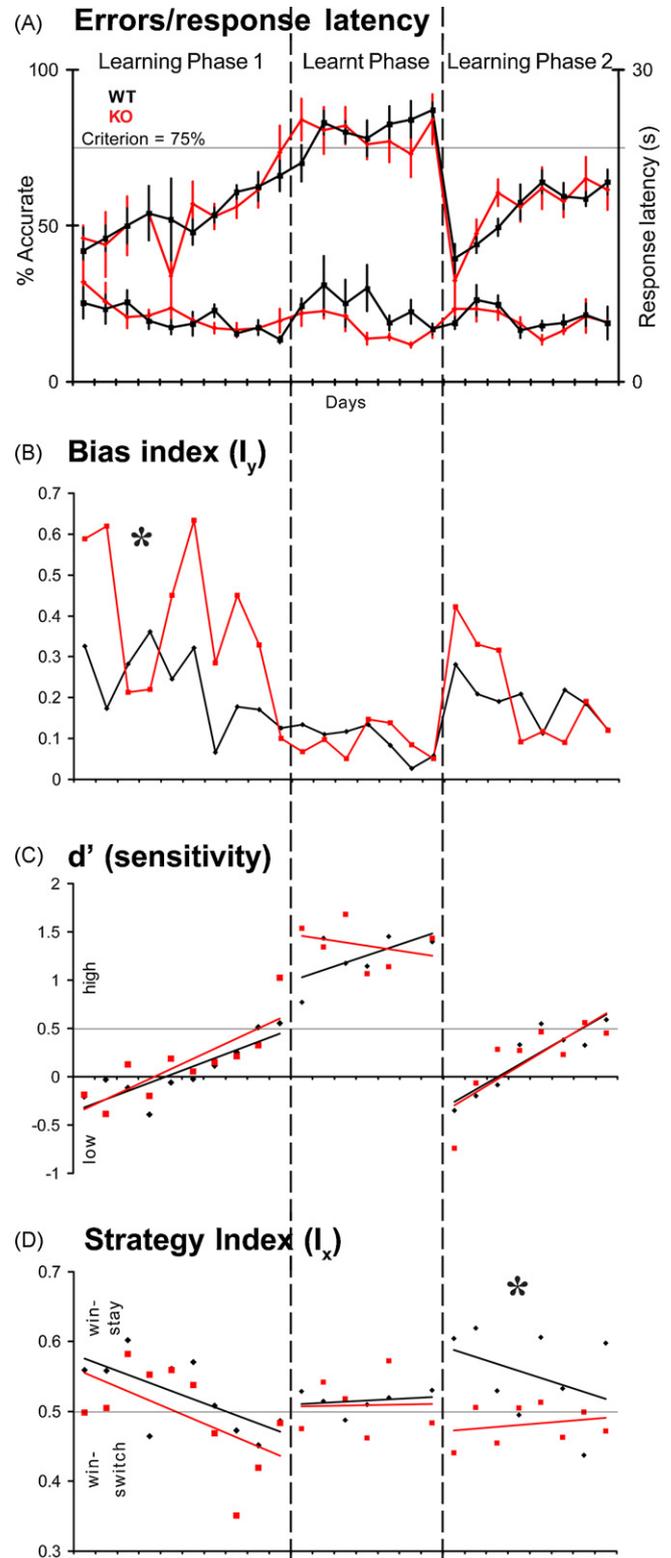


Fig. 1. Graphs showing measurements of behaviour of WT (Black squares) and *ephrin-A2*^{-/-} (red diamonds) mice during a visual discrimination task. Values are separated into three phases: Learning Phase 1 (initial learning to discriminate between two visual stimuli), Learnt Phase (>75% accuracy) and Learning Phase 2 (reversal). (A) Upper lines show the average % of errors for each genotype. Bottom lines show the response latency. (B) Bias index. (C) d' or sensitivity to the stimulus. (D) Strategy index. Significant differences between genotypes for repeated measures across a learning phase are indicated by an asterisk (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.).

3.2. Bias index

All mice had a bias for the left arm of the maze during the learning stages of their training (Fig. 1B). A bias near 0 was present in the Learnt Phase (once mice had reached criterion). During Learning Phase 1, *ephrin-A2*^{-/-} mice demonstrated a stronger bias than WT mice ($F(1, 8)=9.020, P<0.05, \eta_p^2=0.530$), but no difference was detected between genotypes during Learning Phase 2 ($F(1, 8)=0.153, P=0.706$). However, bias significantly decreased from the beginning to the end of the experiment for both genotypes ($t(9)=2.722, P<0.05, r=0.67, \text{Fig. 1B}$).

3.3. Sensitivity to visual stimuli (d')

No pre-existing differences in sensitivity (d') were present between WT ($M=-0.208 \pm 0.342$ SD) and KO mice ($M=-0.053 \pm 0.777$ SD) at the beginning of training ($t(7)=-0.401, P=0.700$; Fig. 1C). For each individual test, the assumption of sphericity was met. During Learning Phase 1, d' increased significantly with time ($F(10, 70)=13.618, P<0.05, \eta_p^2=0.660$), but there was no difference between genotypes ($F(1, 7)=5.013, P=0.060, \eta_p^2=0.417$; Fig. 1C), indicating that both genotypes became more sensitive to the rewarded stimulus with time. No effect of time or genotype was found during the Learnt Phase ($F(4, 432)=0.632, P=0.859$ and $F(1, 8)=0.002, P=0.967$), where sensitivity was extremely high (>1 for all mice in all tests). The different slopes for WT and *ephrin-A2*^{-/-} mice in Fig. 1C during the Learnt Phase are most likely due to individual variation in the time to criterion (see above). During Learning Phase 2, d' followed a similar pattern to that observed in Learning Phase 1, increasing with time as the mice re-learned the task ($F(7, 49)=5.77, P<0.05, \eta_p^2=0.439$), with no effect of genotype ($F(1, 7)=0.149, P=0.711$).

3.4. Strategy index (I_X)

No pre-existing differences in strategy were present between WT ($M=0.559, \text{SD}=0.100$) and KO mice ($M=0.455, \text{SD}=0.137$) at the beginning of training ($t(7)=1.324, P=0.227$; Fig. 1D). During Phase 1, I_X did not change with time ($F(10, 70)=1.800, P=0.076$), nor genotype ($F(1, 7)=3.081, P=0.123$; Fig. 1D). No effect of time or genotype was present during the Learnt Phase ($F(4, 32)=1.320, P=0.284$ and $F(1, 8)=0.012, P=0.917$; Fig. 1D). However, during Learning Phase 2, I_X did not change with time ($F(7, 49)=1.060, P=0.403$), but was significantly different between genotypes ($F(1, 7)=6.801, P<0.05, \eta_p^2=0.493$; Fig. 1D). WT mice reverted to the 'win-stay' strategy employed during Learning Phase 1 ($M=0.549$), particularly in early stages of the phase. In contrast, *ephrin-A2*^{-/-} mice maintained a neutral strategy with weak tendency towards a 'win-switch' strategy ($M=0.482$).

3.5. Bayesian-optimal observer

For the WT group, the best fit of the Bayesian model was obtained with a value of $\eta=1.95$. The correlation between the proportion of subjects that chose the left arm and the Bayesian-optimal decision on each trial was above statistical chance expectations ($r=0.36, P<0.02$) (Fig. 2A). The best fit of the Bayesian model for the *ephrin-A2*^{-/-} group required a value of $\eta=1.01$ that is lower compared to the WT group. The correlation between the observed responses and the Bayesian-optimal decisions was also lower than that obtained for the wild-type group, and did not differ from chance expectations ($r=0.11, P>0.5$) (Fig. 2B). Taken together, these results suggest that, compared with the WT subjects, *ephrin-A2*^{-/-} subjects adapted their decisions less rapidly to a change in stimulus–reward relationship, as evidenced by a lower value of η when fitting the Bayesian model to the observed responses. In addition, the decisions of WT subjects during the first 40 trials of phase II could be described by a Bayesian-optimal observer, while the decisions of *ephrin-A2*^{-/-} subjects could not.

4. Discussion

Here we show that *ephrin-A2*^{-/-} mice have similar learning rates and sensitivity to a stimulus compared to WT mice during the initial acquisition of a visual discrimination task and during reversal learning. Both genotypes employed similar strategies (win-stay) during initial acquisition, but differed during reversal learning: WT mice reverted to a win-stay strategy, returning more frequently to arms where they had just received a reward, whereas *ephrin-A2*^{-/-} mice maintained the neutral strategy that they had developed by the end of the first learning phase.

The similar learning rates and initial strategies suggest normal hippocampal function in *ephrin-A2*^{-/-} mice, because the basic learning and memory components of the task were no different from WT. Furthermore, both genotypes showed a strong bias to the left arm of the Y-maze during learning phases, consistent with previous studies suggesting an innate preference for one side [2]. The most important behavioural difference between the two genotypes was observed during the reversal phase of testing, suggesting that the deficit in *ephrin-A2*^{-/-} mice is likely to involve the orbitofrontal cortex, a region implicated in reversal learning. Previous studies have shown that lesion of the orbitofrontal cortex impairs reversal learning in mice and rats, but does not impact on the initial acquisition of discrimination learning [3]. Furthermore, depletion of serotonin within the OFC shows similar reversal-specific impairments [22].

However, *ephrin-A2*^{-/-} mice do not show a true impairment in reversal learning, because the time to criterion was the same as for WT. Rather, they display a different strategy during reversal. Unlike WT, *ephrin-A2*^{-/-} mice do not return to the win-stay strat-

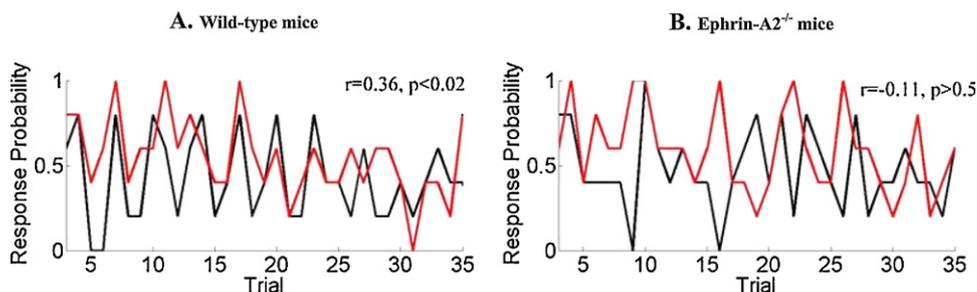


Fig. 2. Bayesian-optimal observer analysis. Graphs show the relationship between the proportion of subjects that chose the left arm (solid black line) and the Bayesian-optimal decision (solid red line) on the first 35 trials (day 1) of Learning Phase 2 for WT mice (A) and *ephrin-A2*^{-/-} mice (B). The Pearson correlation between subjects and the Bayesian-optimal decision are reported above each graph (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article).

egy that they used in the initial learning phase, but rather maintain a neutral strategy that they adopted during the Learnt Phase. The maintenance of a neutral strategy could be seen as a form of perseverance, a behaviour also mediated by the prefrontal cortex [14]. The stronger bias in ephrin-A^{-/-} mice in the initial learning phase is also consistent with stronger perseverance.

Results of a Bayesian analysis were consistent with a perseverance of response strategies in ephrin-A2^{-/-} mice. The Bayesian model that best described the behaviour of ephrin-A2^{-/-} mice during the Learnt Phase had a lower update parameter (η) than the Bayesian model for the WT mice, suggesting that ephrin-A2^{-/-} mice adapted their response strategy more slowly than WT mice. As opposed to WT mice, the response strategy of ephrin-A2^{-/-} mice could not be reliably described by a Bayesian-optimal observer, suggesting that perseverance was not an adaptive learning strategy. Further behavioural studies, for example using a set-shifting paradigm, would identify whether perseverative deficits are present in ephrin-A2^{-/-} mice [14,16].

An alternative view of the behavioural deficit in ephrin-A2^{-/-} mice is that the mice failed to eliminate inappropriate response alternatives. This type of error has been associated with deficits in strategy shifting mediated by the Nucleus accumbens (core; NAc) and can also manifest as a failure to maintain new strategies [8]. The involvement of the NAc is consistent with studies suggesting that behavioural flexibility and choice of strategy is mediated by thalamic–prefrontal cortical–ventral striatal circuitry, with evidence coming from studies in humans [25] and rats [4]. The mediodorsal nuclei of the thalamus (MD), prefrontal cortex (PFC), and nucleus accumbens core (NAc) form an interconnected network that are thought to underlie certain forms of behavioural flexibility, including reversal and set-shifting [4].

mRNA in situ hybridization studies in mouse brain (online Allen Brain Atlas) show that ephrin-A2 is abundantly expressed in the thalamus, prefrontal cortex and nucleus accumbens. Given the role for ephrin-A2 in axonal pathfinding in multiple brain regions, it is possible that the lack of ephrin-A2 might affect the development of these neuronal circuits. The implication is the topographic organisation of projections between these structures may underlie complex behaviours associated with behavioural flexibility [14,30].

Acknowledgements

Supported by Neurotrauma Research Program and Raine Foundation Grants to JR. Professor Leigh Simmons supported the project as part of a student Honours program. A pilot version of this study was carried out by Lina Butenschoen and Christine Hartley. We are grateful for the expert assistance with animal care and breeding from the Animal Care Facilities at UWA.

References

- [1] Allen Mouse Brain Atlas (Internet). Available from: Seattle (WA): Allen Institute for Brain Science; 2009.
- [2] Andrade C, Alwarshetty M, Sudha S, Suresh Chandra J. Effect of innate direction bias on T-maze learning in rats: implications for research. *J Neurosci Methods* 2001;110:31–5.
- [3] Bissonette GB, Martins GJ, Franz TM, Harper ES, Schoenbaum G, Powell EM. Double dissociation of the effects of medial and orbital prefrontal cortical lesions on attentional and affective shifts in mice. *J Neurosci* 2008;28:11124–30.
- [4] Block AE, Dhanji H, Thompson-Tardif SF, Floresco SB. Thalamic–prefrontal cortical–ventral striatal circuitry mediates dissociable components of strategy set shifting. *Cereb Cortex* 2007;17:1625–36.
- [5] Clandinin TR, Feldheim DA. Making a visual map: mechanisms and molecules. *Curr Opin Neurobiol* 2009;19:174–80.
- [6] Cramer KS. Eph proteins and the assembly of auditory circuits. *Hear Res* 2005;206:42–51.
- [7] Feldheim DA, Kim Y-I, Bergemann AD, Frisen J, Barbacid M, Flanagan JG. Genetic analysis of ephrin-A2 and ephrin-A5 shows their requirement in multiple aspects of retinocollicular mapping. *Neuron* 2000;25:563–74.
- [8] Floresco SB, Magyar O. Mesocortical dopamine modulation of executive functions: beyond working memory. *Psychopharmacology (Berl)* 2006;188:567–85.
- [9] Gao PP, Zhang JH, Yokoyama M, Racey B, Dreyfus CF, Black IB, et al. Regulation of topographic projection in the brain: Elf-1 in the hippocamposeptal system. *Proc Natl Acad Sci USA* 1996;93:11161–6.
- [10] Gerlai R, Shinsky N, Shih A, Williams P, Winer J, Armanini M, et al. Regulation of learning by EphA receptors: a protein targeting study. *J Neurosci* 1999;19:9538–49.
- [11] Goodhill G, Richards L. Retinotectal maps: molecules, models and misplaced data. *Trends Neurosci* 1999;22:529–34.
- [12] Green D, Swets J. Signal detection theory and psychophysics. New York: Wiley; 1966.
- [13] Grunwald IC, Korte M, Wolfer D, Wilkinson GA, Unsicker K, Lipp HP, et al. Kinase-independent requirement of EphB2 receptors in hippocampal synaptic plasticity. *Neuron* 2001;32:1027–40.
- [14] Haluk DM, Floresco SB. Ventral striatal dopamine modulation of different forms of behavioral flexibility. *Neuropsychopharmacology* 2009;34:2041–52.
- [15] Haustead D, Lukehurst S, Clutton GB, Dunlop S, Arrese CA, Sherrard RM, Rodger J. Functional topography and integration of the contralateral and ipsilateral retinocollicular projections in ephrin-A^{-/-} mice. *J Neurosci* 2008;28:7376–86.
- [16] Holmes A, Wellman CL. Stress-induced prefrontal reorganization and executive dysfunction in rodents. *Neurosci Biobehav Rev* 2009;33:773–83.
- [17] Hou ST, Jiang SX, Smith RA. Permissive and repulsive cues and signalling pathways of axonal outgrowth and regeneration. *Int Rev Cell Mol Biol* 2008;267:125–81.
- [18] Janis LS, Cassidy RM, Kromer LF. Ephrin-A binding and EphA receptor expression delineate the matrix compartment of the striatum. *J Neurosci* 1999;19:4962–71.
- [19] Kullander K, Butt SJ, Lebrecht JM, Lundfald L, Restrepo CE, Rydstrom A, et al. Role of EphA4 and EphrinB3 in local neuronal circuits that control walking. *Science* 2003;299:1889–92.
- [20] Lackmann M, Boyd AW. Eph, a protein family coming of age: more confusion, insight, or complexity? *Sci Signal* 2008;1:re2.
- [21] Macmillan N, Creelman C. Detection theory: a user's guide. New York: Cambridge University Press; 1991.
- [22] Masaki D, Yokoyama C, Kinoshita S, Tsuchida H, Nakatomi Y, Yoshimoto K, et al. Relationship between limbic and cortical 5-HT neurotransmission and acquisition and reversal learning in a go/no-go task in rats. *Psychopharmacology (Berl)* 2006;189:249–58.
- [23] McLaughlin T, O'Leary DD. Molecular gradients and development of retinotopic maps. *Annu Rev Neurosci* 2005;28:327–55.
- [24] Nomura T, Holmberg J, Frisen J, Osumi N. Pax6-dependent boundary defines alignment of migrating olfactory cortex neurons via the repulsive activity of ephrin A5. *Development* 2006;133:1335–45.
- [25] Paulus MP, Hozack N, Frank L, Brown GG. Error rate and outcome predictability affect neural activation in prefrontal cortex and anterior cingulate during decision-making. *Neuroimage* 2002;15:836–46.
- [26] Prusky G, West P, Douglas R. Behavioral assessment of visual acuity in mice and rats. *Vision Res* 2000;40:2201–9.
- [27] Sahgal A, Clincke GH. A comparison of different methods of assessing patterns of responding in discrete trial choice procedures. *Psychopharmacology (Berl)* 1985;87:374–7.
- [28] Savelieva KV, Rajan I, Baker KB, Vogel P, Jarman W, Allen M, et al. Learning and memory impairment in Eph receptor A6 knockout mice. *Neurosci Lett* 2008;438:205–9.
- [29] Silver L. Mouse genetics: concepts and applications. Oxford: Oxford University Press; 1995.
- [30] Thivierge J-P, Marcus G. The topographic brain: from neural connectivity to cognition. *Trends Neurosci* 2007;30:251–9.
- [31] Vanderhaeghen P, Lu Q, Prakash N, Frisen J, Walsh CA, Frostig RD, et al. A mapping label required for normal scale of body representation in the cortex. *Nat Neurosci* 2000;3:358–65.