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Research report

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

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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 WTs favoured a win-stay strategy, KOs remained relatively neutral. KOs 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.

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, memory 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

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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 cm^2 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:

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

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, $n+1\ldots$) as the repeated measure and Genotype (wild-type or *ephrin-A2*$^{-/-}$) as the between-subjects measure was used to assess d',