P942.04

Lysolecithin (LPC) induced demyelination of the optic chiasm as a model to evaluate remyelinating agents

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NO – PHOTO / RECORD

Introduction

Myelination of axons is necessary for faithful, long-distance conductance of electrical impulses. Multiple sclerosis (MS) is characterized by immune mediated myelin injury and progressive axonal loss. Many patients with MS exhibit myelin-dependent slowing of neuronal transmission in the optic nerve which can be measured through visual evoked potentials (VEP). VEP is a clinically translatable endpoint used to quantify myelin damage in MS patients and in animal models of MS. Demyelination is characterized by a significant latency shift in the VEP waveform signal (Toussaint et al., 1983, Green et al., 2017). While current MS therapies primarily address the immune mediated component of the disease, there has been recent interest in mechanisms that promote remyelination. To evaluate mechanisms that directly impact remyelination, mouse models using acute demyelinating agents, such as, lysolecithin injection into the spinal cord or corpus callosum are used with a histological endpoint to assess the cellular and structural aspects of remyelination. Because of its focal nature, however, attempts at a reliable functional endpoint, such as a behavioral score is difficult to evaluate. While other MS models, like MOG-EAE provide the benefit of both a histological and functional endpoint - the model evaluates compounds that could impact the immune component, oligodendrocyte differentiation, or both. Moreover, the model can at times vary with respect to onset and severity of the demyelination. This model evaluates the myelination component specifically. Recently, a group described a mouse model whereby lysolecithin was directly injected into the optic chiasm (Pourabdolhossein et al., 2014)[1]. Here we validate this model, show a progression from demyelination to remyelination, and importantly, show a correlation between VEP latency and histology across time points. Peak latency delays were seen by 7 days post-lysolecithin (dpl) suggesting an optimal timepoint to evaluate remyelinating compounds. We further show that the VEP delay, and recovery are not caused by differences in inflammation. Because both histological and VEP endpoints can be measured, acute, focal lysolecithin induced demyelination in the optic chiasm provides a rapid, comprehensive method for evaluating remyelinating agents.

Study Design

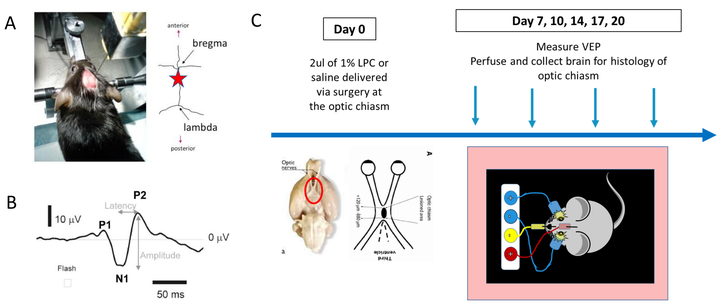

Fig 1A. Mice were anesthetized with Ketamine/xylazine/acepromazine (75/10/2 mg/kg). Demyelination induced by stereotaxic injection of 2µL of 1% lysolecithin (LPC in 0.9% NaCl). Mice positioned in a skull flat situation. LPC was injected into the optic chiasm (red star; 0.5µL/min flow rate; 3.9 mm anterior to Lambda, depth 5.75 mm, zero laterality). Needle kept in place for 5 min to equilibrate tissue and inject solution to avoid reflux through needle tract. Controls injected with equal volume of saline. **1B.** Visual evoked potential (VEP) waveform describing N1 latency, and N1P2 amplitude measurements **1C.** Experimental design with VEP performed at 7, 10, 14, 17, and 20 dpl. Mice anesthetized with ketamine/xylazine (75/10mg/kg) and eyes dilated with 1% tropicamide, 2 minutes. Genteal eyedrops applied for lubrication and mice placed on the Diagnosys Celeris Model D430 (Lowell, MA) heated platform. Electrodes are placed and flash VEP execution. Each exam consists of ≥ 3 runs, with pulse intensity 3 cd.s/m2, 1 Hz, on time 4 ms, pulse color: white-6500K, 100 sweeps per result. Flash VEPs recorded from each eye independently and simultaneously. N1 latency determined by average of N1 from 3 VEP traces

7pl largest acute demyelination effect with recovery at 17-20dpl

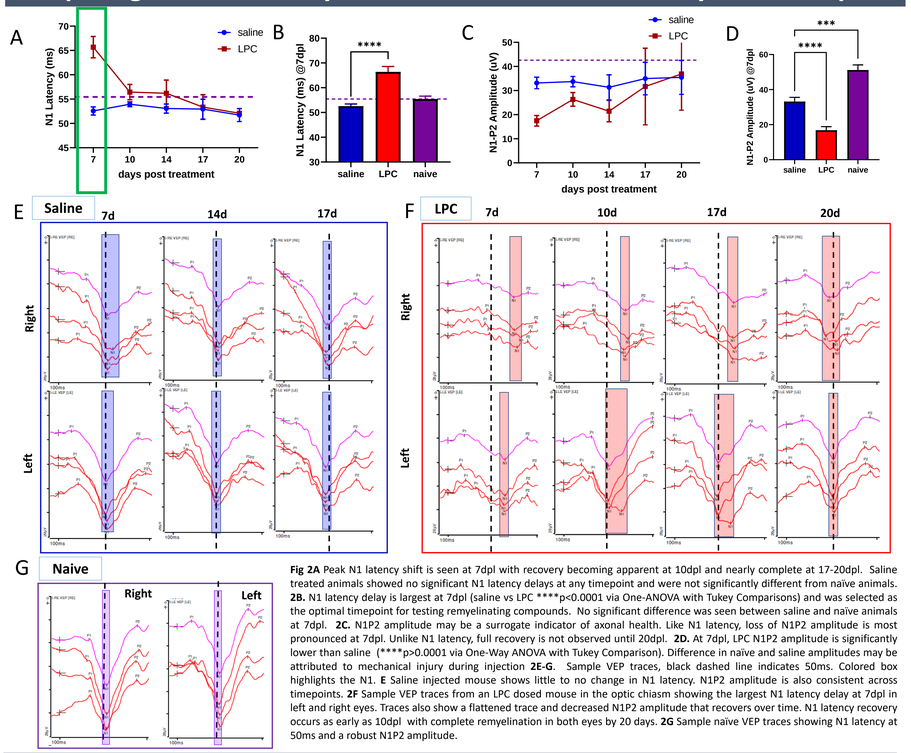

Fig 2A Peak N1 latency shift is seen at 7dpl with recovery becoming apparent at 10dpl and nearly complete at 17-20dpl. Saline treated animals showed no significant N1 latency delays at any timepoint and were not significantly different from naïve animals. **2B.** N1 latency delay is largest at 7dpl (saline vs LPC ****p<0.0001 via One-ANOVA with Tukey Comparisons) and was selected as the optimal timepoint for testing remyelinating compounds. No significant difference was seen between saline and naïve animals at 7dpl. **2C.** N1P2 amplitude may be a surrogate indicator of axonal health. Like N1 latency, loss of N1P2 amplitude is most pronounced at 7dpl. Unlike N1 latency, full recovery is not observed until 20dpl. **2D.** At 7dpl, LPC N1P2 amplitude is significantly lower than saline (****p>0.0001 via One-Way ANOVA with Tukey Comparison). Difference in naïve and saline amplitudes may be attributed to mechanical injury during injection **2E-G**. Sample VEP traces, black dashed line indicates 50ms. Colored box highlights the N1. **E** Saline injected mouse shows little to no change in N1 latency. N1P2 amplitude is also consistent across timepoints. **2F** Sample VEP traces from an LPC dosed mouse in the optic chiasm showing the largest N1 latency delay at 7dpl in left and right eyes. Traces also show a flattened trace and decreased N1P2 amplitude that recovers over time. N1 latency recovery occurs as early as 10dpl with complete remyelination in both eyes by 20 days. **2G** Sample naïve VEP traces showing N1 latency at 50ms and a robust N1P2 amplitude.

Immunohistochemistry of surgical lesions in the optic chiasm

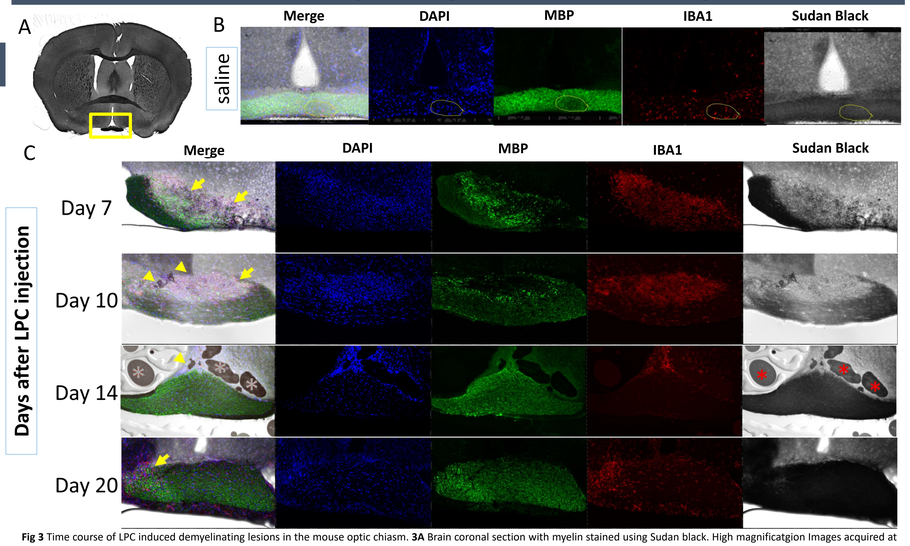

Fig 3 Time course of LPC induced demyelinating lesions in the mouse optic chiasm. **3A** Brain coronal section with myelin stained using Sudan black. High magnificatgion Images acquired at optic chaism (yellow box). **3B** Stained optic chiasm from saline treated mice showing no demyelination occurred. DAPI (nuclear marker, blue), myelin basic protein (MBP; mature myelin marker, green), IBA1 (microglia, red), Sudan black (myelin, brightfield). **3C** Panel of LPC treated mice showing progression in optic chiasm from demyelination to remyelination, day 7 to 20. Yellow arrows, **what are arrowheads?** point to lesion area; **asterisk (gray) artifact**.

Distinct remyelinating mechanisms are efficacious at 7dpl

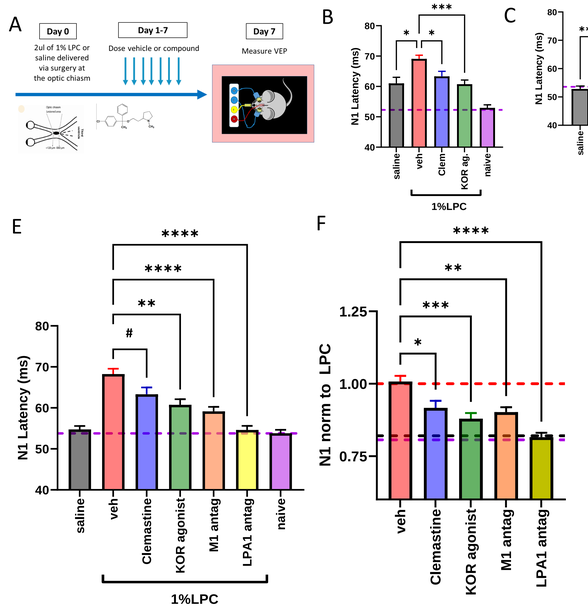

Fig 4A Evaluation of remyelinating agents in acute optic chiasm demyelination model. Drug is given starting 1d after LPC delivery and dosed daily p.o. with VEPs recorded at 7d. **4B-D.** N1 latencies from each experimental cohort **4B.** M1 antagonist Clemastine (10mg/kg) (*p=0.0331 LPC vs Clemastine), and Kappa opioid receptor (**p=0.0010 LPC vs U50,488) show functional remyelination at 7dpl (One-Way ANOVA with Tukey's, *p=0.0143 saline vs LPC) **4C.** In house M1 antagonist (*p=0.0318 LPC vs M1 antag), shows functional remyelination at 7dpl (One-Way ANOVA with Dunnett's, ****p<0.0001 saline vs LPC) **4D.** In house LPA1 antagonist (**p=0.0028 LPC vs LPA1 antag), shows functional remyelination at 7dpl (One-Way ANOVA with Tukey's, **p=0.0038 saline vs LPC) **4E.** Scatterplot of raw N1 latencies from all molecules; #p=0.0393 (LPC vs Clemastine) via t-test; **p=0.0020 (LPC vs KOR agonist), ****p<0.0001 (LPC vs In house M1 antagonist), ****p<0.0001 (LPC vs In house LPA1 antagonist) via One-Way ANOVA with Tukey's. **4F.** Normalized comparison across mechanisms. To account for cohort variability, each drug was normalized to their respective LPC group (red dashed line) and compared to each other. Naïve latency is indicated by the purple dashed line, the saline+veh group is indicated with the black dashed line. *p=0.0223 (LPC vs Clemastine), ***p=0.0007 (LPC vs KOR agonist), **p=0.0050 (LPC vs M1 antagonist in house), ****p<0.0001 (LPC vs LPA1 antagonist in house), One-Way ANOVA with Tukey's.

Conclusions

- We have validated an acute, LPC-induced demyelination model which, in addition to histology, provides the benefit of a rapid functional endpoint.
- The largest functional deficit occurs at 7d post LPC and was chosen as the optimal time to test remyelinating agents. Full recovery via spontaneous remyelination was observed 17-20 days post LPC.
- By histology, N1 latency recovery is not due to reduced inflammation over time
- Unlike MOG-EAE, this model evaluates myelination specifically.
- A diverse set of remyelinating mechanisms (M1, Kappa opioid, and LPA1) show efficacy in the model
- Immunohistochemical quantification of the optic chiasm injection site is challenging to quantify due to the nature of the small size of tissue.

References

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