Research Article

Annals of Behavioral Neuroscience

Dose-Response Relationship of EAE Clinical Severity in a MOG₃₅₋₅₅ Mouse Model: A Pilot Study

Bettenson D, Babatunde S, Gustafson C, Chan R and Metz GAS*

Canadian Centre for Behavioural Neuroscience, Department of Neuroscience, University of Lethbridge, Lethbridge, Canada

***Correspondence:** Gerlinde AS Metz, Canadian Centre for Behavioural Neuroscience, Department of Neuroscience, University of Lethbridge, 4401 University Drive, Lethbridge, AB T1K 3M4, Canada, E-mail: gerlinde.metz@uleth.ca

Received: August 08, 2018; Accepted: September 31, 2018; Published: November 04, 2018

Abstract

Experimental Autoimmune Encephalomyelitis (EAE) is a common animal model of Multiple Sclerosis (MS) which mimics the main autoimmune, demyelinating, and inflammatory hallmarks of this human disorder. To better understand the severity of the symptoms in relation to the antigen in EAE, we explored the dose-symptom relationship between the quantity of myelin oligodendrocyte glycoprotein (MOG₃₅₋₅₅) and clinical symptoms in a C57/BL6 mouse pilot study. To isolate the impact of MOG₃₅₋₅₅ we developed an EAE model that does not require the additional application of pertussis toxin (virulence factor of *Bordetella pertussis*). Mice were treated with either 50 µg, 100 µg, or 150 µg of MOG₃₅₋₅₅ emulsified in complete Freund's adjuvant. Following induction, the mice were assessed for clinical symptoms daily, and assessed weekly for gross and fine motor impairments, mechanical allodynia, and anxiety-like behaviours for eight weeks. The time course of sensorimotor function loss was characterized by multiphasic disease progression. Findings also suggested an inverted U-shape dose-response relationship with a medium dosage of 100 µl MOG₃₅₋₅₅ dosage aggravating symptom severity in induced animals. Outcomes measured by a clinical score correlated with performance on motor and nociceptive threshold tasks. As the disease progressed, fine and gross motor impairments and nociceptive threshold increased and impairments persisted beyond eight weeks. This study indicates that mild to moderate EAE can be induced in the absence of use of pertussis toxin. The progression suggests a spontaneously multiphasic disease course, which may have attractive implications for clinically relevant animal models.

Keywords: Experimental autoimmune encephalomyelitis, EAE, Multiple sclerosis, Dose-response relationship, Clinical symptoms, Motor function, Skilled movement, Recovery, Stress

Introduction

Multiple Sclerosis (MS) is an autoimmune disease of the Central Nervous System (CNS). It is characterized by chronic inflammation of the CNS which is initiated by the activation of auto-reactive, myelin-specific T cells in the periphery [1]. The T-cells permeate the blood-brain barrier (BBB) to invade the brain and spinal cord [2],[3]. Here, they trigger a cascade of events that promote

local inflammation and demyelination culminating in axonal loss and eventually cell death [4]. These neurodegenerative processes are accompanied by functional loss, including sensory and motor impairments [5-7]. The functional decline in patients differentiates two main types of disease progression. Primary progressive MS is characterized by a steady decline in function where as in relapsing-remitting MS patients experience bouts of impairments and disease remissions [8]. The

relapsing-remitting progression can potentially develop into a secondary progressive disease course, in which a steady functional decline occurs, much like the primary progressive disease course [8].

The most widely used animal model to study MS pathology and clinical symptoms is based on experimental autoimmune encephalomyelitis (EAE) in rodents [9],[10]. By inducing autoimmunity to a myelin-specific antigen, the EAE model reproduces some of the hallmark neuropathological and behavioural features of human MS [11-13]. Antigens used for EAE induction in rodents include purified myelin, myelin proteins such as myelin basic protein (MBP), proteolipid protein (PLP₁₃₉₋₁₅₁), myelin-associated glycoprotein (MAG), and myelin oligodendrocyteglycoprotein (MOG₃₅₋₅₅) [14]. When systemically injected into the animal, they stimulate an immune response to proliferate and expand activated immune cells directed against the antigen [15]. The typically monophasic course of EAE usually begins with tail weakness or paralysis, followed by hind limb impairment and paraplegia, then forelimb impairment and even quadriplegia in extreme cases [16-18]. The present study used female mice as they generally displays wider range of symptoms than males. These include exacerbated paralysis, the presence of neuropathic pain, and reduced remyelination during the remission phase [19],[20].

The technique commonly used to induce the EAE utilizes a subcutaneous injection of the peptide MOG₃₅₋₅₅ emulsified in Complete Freund's Adjuvant (CFA), mineral oil and *Mycobacterium tuberculosis* strain H37RA [13],[18]. A standardized dose of MOG₃₅₋₅₅ in association with clinical outcomes in an EAE mouse models has not been established yet. Here we performed a dose-response pilot study in female C57BL/6 mice using a comprehensive behavioural test battery designed to capture subtle functional changes to isolate the impact of MOG₃₅₋₅₅ we developed an EAE model that does not require the additional application of pertussis toxin. The underlying hypothesis of this study is that the time course of fine motor skills, sensorimotor function, nociception, fatigue, anxiety-and depression-like behaviors is influenced by the MOG₃₅₋₅₅ dosage.

Methods
Animals

Nine female, naïve C57/BL6 mice, 60 days old at the beginning of the experiment, were used. The animals were housed in groups of two or three under standard environmental conditions (12:12 hour light/dark cycle Annal Behav Neurosci, 1(1): 123-132 (2018)

with lights on at 7:30 AM). All experimental procedures were performed in accordance with the guidelines of the Canadian Council on Animal Care and approved by the institutional Animal Care Committee of the University of Lethbridge.

EAE induction

Prior to the injection, baseline weights were recorded. At the age of 60 days, three mice each were assigned to one of the following dosage groups: EAE induction via hind flank subcutaneous injection of either 50 µL, 100 µL, or 150 µL of 1 mg/ml solution of MOG₃₅₋₅₅ emulsified in CFA as per [18]. Following EAE induction, the animals were allowed two days of rest and recovery. Based on successful pilot experiments, this model did not involve the use of pertussis toxin.

EAE symptom monitoring

Mice were weighed daily and assessed for clinical symptoms using a 5-point scoring system [16],[18] to monitor disease progression and recovery following EAE (Table 1). Tail weakness was rated as 1 point, paralysis received 3 points, quadriplegia received 4 points and death was rated as 5 points. These assessments were performed daily up to eight weeks following EAE induction.

Table 1: The five-point scoring scale used to monitor the progression of EAE with higher scores indicating more severe paralysis.

Clinical Score	Symptoms
0	Asymptomatic
0.5	Partial tail paralysis
1	Full tail paralysis
2	Hind limb weakness and waddling gait
2.5	Single hind limb paralysis and waddling gait
3	Full hind limb paralysis
4	Quadriplegia
5	Death

Behavioural test battery

The following behavioural tests were performed prior to EAE induction and weekly for eight weeks post-induction [21].

Open field exploration test: Mice were placed individually in a circular open field arena for a 5-minute free exploration trial. During each trial, the exploratory

pathways of mice were digitally tracked (HVS Image Ltd., Buckingham, England). The HVS software calculated the total path length (-cm) travelled during the trial, the average speed (-cm/s), and the x and y coordinates to recreate a travel path [22],[23].

Ladder rung walking task: Animals were trained to cross a custom-made horizontal ladder rung apparatus from a neutral starting point to an enclosed shelter goal in accordance to procedures described by Metz and Whishaw (2009, 2002) [24],[25]. The ladder rung walking apparatus consisted of two Plexiglas side walls and metal rungs (3mm in diameter) which were inserted to create a floor with a minimum distance of 1cm between rungs. The following day after baseline training and each post-induction week, three trials per animal were recorded and scored based on Metz and Whishaw (2009, 2002) [24],[25]. In short, this scoring method provides qualitative measurements of foot and limb placement of the rungs and quantitative evaluation of number of rung misses, slips, and successful placements.

Von Frey hair test: Using Von Frey hair filaments of increasing gauge, a paw withdrawal response to a pressure stimulus was elicited in the hind paws of each animal to measure nociception sensitivity [21],[26]. Before each set of trials, animals were placed in an elevated testing compartment and allowed 15-30 minutes to explore and groom until they were relaxed and stationary. Once stationary, pressure was applied with the filament to the bottom of each animal's hind paw to elicit a withdrawal response to the nociceptive stimulus. If no response was observed, the gauge of filament was increased until a paw withdrawal response was elicited, or until a maximum of 15-gauge filament was reached without a response, indicating an absence of pain sensation in that paw. Animals were habituated to the chamber for 10 minutes prior to testing, and given a 5 minute rest period between trials.

Elevated plus maze: Mice were placed individually at the centre of an elevated plus-shaped maze with two open and two enclosed arms (50 cm long by 10 cm wide) for a 5-minute exploration session. Trials were video recorded and videos were scored for total dwell time spent in the open and closed arms, and time spent in the open arms [27],[28]. In addition, time spent risk assessing was also recorded.

Statistical analysis

Statistical analyses of behavioural data included of a

repeated measures ANOVA across baseline and post-induction weeks, followed by Fisher's Least Significant Difference (LSD) pair wise comparisons. Two sets of this analysis were performed, one with a between subjects factor of MOG₃₅₋₅₅ solution dosage, and the second with all dosage groups merged into one data set. A Pearson's correlation analysis was also performed to correlate the clinical scores with performance in each behavioural task. The threshold considered significant was $p < 0.05$.

Results

Clinical symptoms revealed an effect of the moderate MOG₃₅₋₅₅ Dose

Clinical symptoms in EAE mice ranged from tail weakness in the mild cases, to full hind limb paralysis combined with forelimb impairment. There were no differences in disease progression and symptom severity between different dosages (Figure 1A). Five mice experienced two or more attacks with increased paralysis symptoms, and four of the mice experienced a single hit of paralysis symptoms. The peak of symptoms occurred between day 18-23 post-induction. Recurrent attacks occurred around post-induction day 35 and 50. At the peak of symptoms during post-induction week 3, animals in each group exhibited hind limb impairment or paralysis. Animals in the 100 μ L MOG₃₅₋₅₅ dose group exhibited a tendency for forelimb impairment in addition to hind limb paralysis. Figure 1B illustrates the average multiphasic time course of EAE symptoms across groups. The findings suggest an inverted U-shape dose-response relationship with a medium dosage of 100 μ L MOG₃₅₋₅₅ accelerating the course of EAE and aggravating symptom severity in induced animals.

Behavioural outcomes reveal EAE severity but not MOG₃₅₋₅₅ Dose

Open field test: Open field exploratory locomotor activity showed an effect of Post-induction Week on path length in EAE (ANOVA, $p = 0.005$). However, there was no effect of MOG₃₅₋₅₅ dose on the total path length traveled across the post-induction weeks (ANOVA, $p = 0.575$; Figure 2A). Compared to a pooled mean baseline path length of 90cm, the mean path length was reduced to 50.5cm during the peak of EAE symptoms in post-induction week 3 (Figure 2B). The gait impairments persisted until the final day of observation during post-induction week 8, at which animals on average travelled a path length of 56.3cm. Pair wise comparisons of the total path length in baseline measurements before EAE induction and each post-induction week regardless of MOG₃₅₋₅₅ dose revealed

Figure 1: EAE clinical symptoms.

(A) Clinical scores for three mice groups based on MOG dosage for EAE induction. The 50 μ L (Red), 100 μ L (Blue), 150 μ L (Green) dosage groups were monitored from post-induction day 1 to post-induction day 56. ($n=3$ / group). (B) Mean clinical scores compiled across the three MOG dosage groups after EAE induction. Asterisk indicates significance: * $p < 0.05$.

that the total path length was shorter than the baseline measurements (Fisher's LSD, $p < 0.01$).

Ladder rung walking task: Skilled motor impairments of paw placement accuracy and inter-limb coordination were recorded by observing the placement of fore- and hind paws while traversing the horizontal ladder. There was no significant effect of MOG₃₅₋₅₅ Dose on fore- or hind limb placement (Figure 3A) and error scores (Figure 3B) across the post-induction weeks (Placement: ANOVA, $p = 0.583$; Errors: ANOVA, $p = 0.598$). Furthermore, there was no effect of Post-induction Week on fore- or hind limb placement and errors (Placement: ANOVA, $p = 0.206$; Error: ANOVA, $p = 0.169$). However, hind limb error scores during post-induction Week 8 were significantly higher than hind limb error scores at baseline (ANOVA, $p = 0.038$), post-induction week 1 (ANOVA, $p = 0.049$), and post-induction week 2 (ANOVA, $p = 0.039$) measurements (Figure 3B).

Figure 2: Horizontal exploratory activity.

(A) Distance traveled by mice during open field exploration as a function of MOG₃₅₋₅₅ dosage. Baseline measurements were taken prior to induction and further testing occurred weekly up to eight weeks after induction. (B) Mean travel distance was compiled across the three MOG dosage groups after EAE induction. Reduced locomotor activity on the ground indicates a change in motivation, anxiety, and/or loss of motor ambulatory capacity. Asterisks indicate significances compared to baseline: ** $p < 0.01$.

= 0.039) measurements (Figure 3B).

Von Frey hair test: When quantifying the mechanical nociception sensitivity in mice using Von Frey hair filaments, both hind limbs showed a significant change in nociception sensitivity over time (Right limb: ANOVA, $p = 0.007$; Left limb: ANOVA, $p = 0.028$; data not shown). Despite of a trend for exacerbated pain sensitivity in the moderate 100 μ L dose, no effect of MOG₃₅₋₅₅ dose (Figure 4A and B) was observed (Right limb: ANOVA, $p = 0.714$; Left limb: ANOVA, $p = 0.88$). In addition, pooled pair-

wise comparisons (Figure 4C and D) revealed that both right and left forelimbs exhibited a significant increase in sensitivity following post-induction week 1 when compared to the pre-induction baseline measurements (ANOVA, $p < 0.05$).

Figure 3: Skilled walking ability.

(A) Number of foot placement accuracy in the ladder rung walking task across the three MOG concentration groups after EAE induction. Fore- and hind limb placements were combined. (B) Number of foot placement errors calculated across three MOG concentration groups after EAE induction. Note that the foot placement score was more sensitive to display EAE clinical impairments. Mice were first evaluated at baseline prior to induction, then every week post-induction for duration of eight weeks. Asterisks indicate significances: * $p < 0.05$.

Elevated plus maze: Anxiety-related behaviours were reflected in closed arm dwell time, open arm dwell time, and risk assessment time. There was no a significant effect of Time or MOG₃₅₋₅₅ dose on open arm dwell time (Week: ANOVA, $p = 0.384$; Condition: ANOVA, $p = 0.713$; Figure 5A). Similarly, there was no significant effect of Time or Dosage on closed arm dwell time (Week: ANOVA, $p = 0.432$; Condition: ANOVA, $p = 0.564$), and risk assessment time (Week: ANOVA, $p = 0.492$; Condition: ANOVA, $p = 0.547$). Merged data analysis (Figure 5B) revealed a significant decrease in open arm dwell time

during post-induction week 1 compared to baseline measurements ($p < 0.05$).

Correlation Analysis

Correlation analysis confirmed a close relationship between clinical scores and behavioural outcomes. In the open field test, the total path length travelled was negatively correlated with the clinical score assigned on the corresponding test day (Figure 6A; 2-tailed Pearson's correlation, $P = -0.590$, $p < 0.001$). Thus, animals with the lowest clinical scores also travelled less, either by reduced motivation, higher anxiety-like status or by reduced ambulatory capacity.

The Von Frey hair test results showed a positive correlation between clinical score and the minimum filament gauge that elicited a paw withdrawal response in both hind limbs (Figure 6B; 2-tailed Pearson's correlation, $P = 0.805$, $p < 0.001$). Thus, animals with the highest clinical score also showed the most responsiveness to nociceptive stimulation.

Figure 4: Mechanical allodynia.

(A) and (B) show the minimum Von Frey hair gauge needed elicit a withdrawal response from the right and left hindlimb, respectively in the 50 μ L (red), 100 μ L (blue), and 150 μ L (green) MOG concentration groups.

(C) and (D) show a pooled mean from all groups of the same measurement in the right and left hindlimb respectively. A shorter latency represents higher pain sensitivity. Asterisks indicate significances: * $p < 0.05$.

Based on the animal's performance on the ladder rung walking task, the error score (Figure 6C; 2-tailed Pearson's correlation, $P = -0.554$, $p < 0.001$) and the hind limb placement score (Figure 6D; 2-tailed Pearson's correlation, $P = -0.612$, $p < 0.001$) were correlated with the clinical score for the corresponding test day. Thus, mice with the highest clinical score also made more limb placement errors and had the most difficulty with accurate limb placement and inter-limb coordination. There was no correlation between open arm dwell time in the elevated plus maze (2-tailed Pearson's correlation, $P = -0.095$, $p = 0.440$) and the time spent in risk assessment (2-tailed Pearson's correlation, $P = -0.030$, $p = 0.800$) with the corresponding daily clinical scores.

Figure 5: Anxiety-like behaviours.

(A) Time spent in open arms of the elevated plus maze among 50 µL (red), 100 µL (blue), and 150 µL (green) dosage groups. (B) Amount of time spent dwelling on the open arms for all dosage groups combined. Shorter time on the open arm indicates higher levels of anxiety-like behaviour. Baseline measurements were taken prior to EAE induction and further testing occurred every week for eight weeks after induction. Note that the acute stage of EAE was characterized by the lowest dwell time. Asterisk indicates significance: * $p < 0.05$.

Discussion

The present pilot data present a modified version of the standard EAE mouse model, which was characterized by multiphasic disease progression. In general, the main symptoms revealed a mild dose-independency with symptoms correlating with performance in motor and nociceptive threshold tests. The medium dosage of 100 µL MOG₃₅₋₅₅ dosage in particular accelerated the course of EAE. As the disease progressed, fine and gross motor impairments diminished and nociceptive threshold increased. Animals induced with this model also presented with clinical and behavioural impairments persisting beyond eight weeks, indicating permanent tissue loss and insufficient remyelination.

Figure 6: Predictive value of behavioural tests for EAE clinical scores.

Pearson's correlation plots of any post-induction day were compared to clinical scores.

(A) open field path lengths (distance travelled), (B) minimum Von Frey hair gauges that elicited paw withdrawal, and ladder rung walking task hind limb error scores (C) and limb placement scores (D). All animal data was pooled into one dataset for this analysis. Note that reduced path length, lower nociceptive threshold, more limb placements errors and poorer limb placement on the rungs was related to lower EAE clinical severity. Asterisks indicate significances: ** $p < 0.01$.

The present pilot data confirm the expectation that clinical symptoms in the EAE model are dose-dependent.

Published EAE induction protocols utilized a dosage of 200 µg of MOG₃₅₋₅₅ [12],[13], and considerable deviations from this norm (± 50-150 µg) in combination with varying dosages of pertussis toxin [18]. It is important to recognize the severe comorbidities potentially caused by higher MOG doses that would obscure any behavioural results and their interpretation due to confounding pain and inflammation. Therefore, finding the optimal dosage and characterize or refute the dose-response curve of the EAE model is imperative.

The present study serves as a proof-of-principle study to establish that EAE-like symptoms can be induced in the absence of pertussis toxin. The major body of previous record used pertussis toxin to promote blood-brain-barrier (BBB) permeability [29-32] lasting as long as 24 hours. However, a study by Bennet et al. [33] theorizes that BBB degradation requires that pertussis toxin is used in conjunction with the pathogen-associated factors and widespread inflammation. Therefore, the lack of pertussis toxin may have affected our model to become less predictable in terms of EAE progression, either relapsing remitting (RR) or monophasic.

The current mouse test battery assessed correlates to human clinical symptoms including impaired fine and gross motor function, reduced ambulatory capacity [34],[35], altered nociceptive threshold in affected limbs [17],[35],[36], and increased anxiety- and depressive-like behaviours [37],[38]. The present data agree with these previous findings in EAE mouse and rat models, however, without the use of pertussis toxin the functional loss was comparatively mild. Nevertheless, the comprehensive behavioural test battery was particularly designed to display even subtle motor deficits and EAE-associated comorbidities, thus revealing functional changes where other attempts failed to show symptoms [39]. Presently, mice induced with EAE initially experienced a reduced nociceptive threshold in hind limbs in response to pressure from a Von Frey hair filament [35],[40]. As the disease progressed, mice experienced gross and fine motor impairments in tests such as the open field exploration task [35] and ladder rung walking task [41], respectively. Mice that made more errors crossing the horizontal ladder rung can be expected to have more severe paralysis, impaired inter-limb coordination and/or interrupted cortical motor control than those whom experienced fewer errors. While the elevated plus maze task did not indicate a clear dose-response relationship with MOG₃₅₋₅₅ in anxiety- and depressive-like behaviours, it showed that acute EAE symptoms within the first week

most severely affect anxiety-like behaviours. Shorter dwell time in open arms generally indicates anxiogenic consequences of EAE [27],[28]. At later time points, it is possible that severe sensorimotor function loss may have overshadowed emotional changes. Following a peak in symptoms (day 18-23 post-induction), the animals improved in the continuously monitored functional assessments. The extent of recovery generally depends on the severity of the disease, as with a more extensive degree of demyelination and neuronal degeneration EAE may result in residual impairments [13], [18].

It is possible that symptomatic changes in the EAE model may be related to nociceptive or emotional comorbidities [42]. For example, the decreased nociceptive threshold observed at the beginning of the tests may impact sensorimotor function and the performance in skilled and non-skilled motor tasks. It is also possible that emotional changes such as anxiety- and depression-like symptoms or even stress-related emotional disturbances influenced motor function (Metz, 2007) and nociceptive sensitivity. Both anxiety-like responses and depression-like behaviours have been linked to decreased nociceptive threshold in rodents [43],[44]. In addition, acute and chronic stress have been shown to lead to temporary alteration at the nociceptive threshold [45-47] and ultimately exacerbate neuropathic pain [43],[48]. While it is challenging to disentangle the interactions of emotional changes and stress response in the EAE model, it is likely that the origin of the observed sensorimotor responses in the present study has a multifactorial background.

One potential factor that affects an animal's susceptibility to EAE may be the impact of stress [21]. For example, shipment stress experienced during their transport from the external breeder to the testing facility may enhance the vulnerability of the immune system to EAE induction [30]. Previous research has shown that stress during pre-adolescent life can have significant effects on susceptibility to autoimmune disorders as a result of altered immune function brought on by stress [49]. Stressful experiences at any time in life may potentially affect the validity of studies that examine stress effects on development and disease [29],[49]. The high induction rate in the present study therefore may be the result of confounding stressful experiences such as shipment that made them more susceptible to EAE.

In summary, the present pilot study indicated that mild to moderate EAE can be induced in the absence of use of pertussis toxin. While the small sample size of the present

study limits the data interpretation, the overall course of clinical and behavioural symptoms indicates an inverted U-shape dose-response relationship with a medium dosage of 100 µl MOG₃₅₋₅₅ dosage aggravating symptom severity in induced animals. Moreover, the progression of the symptoms suggests a spontaneously multiphasic disease course, which addresses the current need for multiphasic animal models of MS with less predictable clinical outcomes.

Data availability

The original video data used to support the findings of this study are available from the corresponding author upon request.

Acknowledgements

This research was supported by the Alberta Innovates-Health Solution Collaborative Research and Innovation Opportunities program (V. Wee Yong, GM), the Natural Sciences and Engineering Research Council of Canada (GM), and the Alberta MS Network (GM).

References

1. Huseby E, Liggitt D, Brabb T, Schnabel B, Öhlén C, Goverman J. A pathogenic role for myelin-specific Cd8(+) T cells in a model for multiple sclerosis. *J Exp Med*. 2001;194(5):669-676. doi: <https://doi.org/10.1084/jem.194.5.669>
2. Juhler M, Barry DI, Offner H, Konat G, Klinken L, Paulson OB. Blood-brain and blood-spinal cord barrier permeability during the course of experimental allergic encephalomyelitis in the rat. *Brain Res*. 1984;302(2):347-355. doi: [https://doi.org/10.1016/0006-8993\(84\)90249-X](https://doi.org/10.1016/0006-8993(84)90249-X)
3. Petty MA, Lo EH. Junctional complexes of the blood-brain barrier: permeability changes in neuroinflammation. *Prog Neurobiol*. 2002;68(5):311-323. doi: [https://doi.org/10.1016/S0301-0082\(02\)00128-4](https://doi.org/10.1016/S0301-0082(02)00128-4)
4. McQualter JL, Bernard CCA. Multiple sclerosis: a battle between destruction and repair. *J Neurochem*. 2007;100(2):295-306. doi: <https://doi.org/10.1111/j.1471-4159.2006.04232.x>
5. Lucchinetti CF, Bruck W, Lassmann H. Evidence for pathogenic heterogeneity in multiple sclerosis. *Ann Neurol*. 2004;56(2):308-308. doi: <https://doi.org/10.1002/ana.20182>
6. Lassmann H, Bruck W, Lucchinetti CF. The

immunopathology of multiple sclerosis: an overview. *Brain Pathology*. 2007;17(2):210-218. doi: <https://doi.org/10.1111/j.1750-3639.2007.00064.x>

7. Lublin FD, Reingold SC, Cohen JA, et al. Defining the clinical course of multiple sclerosis: the 2013 revisions. *Neurol*. 2014;83(3):278-286. doi: <https://doi.org/10.1212/WNL.0000000000000560>
8. Goldenberg MM. Multiple sclerosis review. *Pharm Therap*. 2012;37(3):175-184. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3351877/>
9. Rivers TM. Encephalomyelitis accompanied by myelin destruction experimentally produced in monkeys. *J Exp Med*. 1935;61(5):689-702. doi: <https://doi.org/10.1084/jem.61.5.689>
10. Mix E, Meyer-Rienecker H, Hartung H-P, Zettl UK. Animal models of multiple sclerosis-Potentials and limitations. *Prog Neurobiol*. 2010;92(3):386-404. doi: <https://doi.org/10.1016/j.pneurobio.2010.06.005>
11. Amor S, Groome N, Linington C, et al. Identification of epitopes of myelin oligodendrocyte glycoprotein for the induction of experimental allergic encephalomyelitis in SJL and Biozzi AB/H mice. *J Immunol*. 1994;153(10):4349-4356.
12. Miller SD, Karpus WJ, Davidson TS. Experimental autoimmune encephalomyelitis in the mouse. *Curr Protoc Immunol*. 2010;Chapter 15:Unit 15.1. doi: <https://doi.org/10.1002/0471142735.im1501s77>
13. Bittner S, Afzali AM, Wiendl H, Meuth SG. Myelin oligodendrocyte glycoprotein (MOG₃₅₋₅₅) induced experimental autoimmune encephalomyelitis (EAE) in C57BL/6 mice. *J Vis Exp*. 2014;(86). doi: <https://doi.org/10.3791/51275>
14. Gold R, Hartung H-P, Toyka KV. Animal models for autoimmune demyelinating disorders of the nervous system. *Mol Med Today*. 2000;6(2):88-91. doi: [https://doi.org/10.1016/S1357-4310\(99\)01639-1](https://doi.org/10.1016/S1357-4310(99)01639-1)
15. Mohajeri M, Sadeghizadeh M, Javan M. Pertussis toxin promotes relapsing-remitting experimental autoimmune encephalomyelitis in Lewis rats. *J Neuroimmunol*. 2015;289:105-110. doi: <https://doi.org/10.1016/j.jneuroim.2015.10.012>
16. Munoz JJ, Bernard CCA, Mackay IR. Elicitation of experimental allergic encephalomyelitis (EAE) in mice with the aid of pertussigen. *Cell Immunol*. 1984;83(1):92-100. doi: [https://doi.org/10.1016/0008-8749\(84\)90228-4](https://doi.org/10.1016/0008-8749(84)90228-4)
17. Olechowski CJ, Truong JJ, Kerr BJ. Neuropathic pain behaviours in a chronic-relapsing model of

- experimental autoimmune encephalomyelitis (EAE): *Pain*. 2009;141(1):156-164. doi: <https://doi.org/10.1016/j.pain.2008.11.002>
18. Berard JL, Wolak K, Fournier S, David S. Characterization of relapsing-remitting and chronic forms of experimental autoimmune encephalomyelitis in C57BL/6 mice. *Glia*. 2009;58(4):434-445. doi: <https://doi.org/10.1002/glia.20935>
19. Papenfuss TL, Rogers CJ, Gienapp I, et al. Sex differences in experimental autoimmune encephalomyelitis in multiple murine strains. *J Neuroimmunol*. 2004;150(1-2):59-69. doi: <https://doi.org/10.1016/j.jneuroim.2004.01.018>
20. Rahn EJ, Iannitti T, Donahue RR, Taylor BK. Sex differences in a mouse model of multiple sclerosis: neuropathic pain behavior in females but not males and protection from neurological deficits during proestrus. *Biol Sex Differ*. 2014;5(1):4. doi: <https://doi.org/10.1186/2042-6410-5-4>
21. Gerrard B, Singh V, Babenko O, et al. Chronic mild stress exacerbates severity of experimental autoimmune encephalomyelitis in association with altered non-coding RNA and metabolic biomarkers. *Neurosci*. 2017;359:299-307. doi: <https://doi.org/10.1016/j.neuroscience.2017.07.033>
22. Crawley JN. Exploratory behavior models of anxiety in mice. *Neurosci. Biobehav. Rev*. 1985;9(1):37-44. doi: [https://doi.org/10.1016/0149-7634\(85\)90030-2](https://doi.org/10.1016/0149-7634(85)90030-2)
23. Faraji J, Soltanpour N, Jafari SY, et al. Stress inhibits psychomotor performance differently in simple and complex open field environments. *Horm Behav*. 2014;65(1):66-75. doi: <https://doi.org/10.1016/j.yhbeh.2013.11.007>
24. Metz GA, Whishaw IQ. The ladder rung walking task: A scoring system and its practical application. *J Vis Exp*. 2009;(28):1204. doi: <https://doi.org/10.3791/1204>
25. Metz GA, Whishaw IQ. Cortical and subcortical lesions impair skilled walking in the ladder rung walking test: a new task to evaluate fore- and hindlimb stepping, placing, and co-ordination. *Journal of Neuroscience Methods*. 2002;115(2):169-179. doi: [https://doi.org/10.1016/S0165-0270\(02\)00012-2](https://doi.org/10.1016/S0165-0270(02)00012-2)
26. von Frey M. Über den Gebrauch von Reizhaaren. Untersuchungen über die Sinnesfunctionen der menschlichen Haut. Erste Abhandlung: Druckempfindung und Schmerz. Abhandlungen der mathematisch-physischen Classe der Königlich Sächsischen Gesellschaft der Wissenschaften. 1896;23:208-217.
27. Komada M, Takao K, Miyakawa T. Elevated plus maze for mice. *J Vis Exp*. 2008;(22). doi: <https://doi.org/10.3791/1088>
28. Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods*. 1985;14(3):149-167. doi: [https://doi.org/10.1016/0165-0270\(85\)90031-7](https://doi.org/10.1016/0165-0270(85)90031-7)
29. Linthicum DS, Munoz JJ, Blaskett A. Acute experimental autoimmune encephalomyelitis in mice. *Cell Immunol*. 1982;73(2):299-310. doi: [https://doi.org/10.1016/0008-8749\(82\)90457-9](https://doi.org/10.1016/0008-8749(82)90457-9)
30. Olfe J, Domanska G, Schuett C, Kiank C. Different stress-related phenotypes of BALB/c mice from in-house or vendor: alterations of the sympathetic and HPA axis responsiveness. *BMC Physiology*. 2010;10(1):2. doi: <https://doi.org/10.1186/1472-6793-10-2>
31. Yong T, Meininger GA, Scott Linthicum D. Enhancement of histamine-induced vascular leakage by pertussis toxin in SJL/J mice but not BALB/c mice. *J Neuroimmunol*. 1993;45(1-2):47-52. doi: [https://doi.org/10.1016/0165-5728\(93\)90162-R](https://doi.org/10.1016/0165-5728(93)90162-R)
32. Kugler S, Bocker K, Heusipp G, Greune L, Kim KS, Schmidt MA. Pertussis toxin transiently affects barrier integrity, organelle organization and transmigration of monocytes in a human brain microvascular endothelial cell barrier model. *Cell Microbiol*. 2007;9(3):619-632. doi: <https://doi.org/10.1111/j.1462-5822.2006.00813.x>
33. Bennett J, Basivireddy J, Kollar A, et al. Blood-brain barrier disruption and enhanced vascular permeability in the multiple sclerosis model EAE. *J Neuroimmunol*. 2010;229(1-2):180-191. doi: <https://doi.org/10.1016/j.jneuroim.2010.08.011>
34. Calabresi PA. Diagnosis and management of multiple sclerosis. *Am Fam Physician*. 2004;70(10):1935-1944.
35. Thibault K, Calvino B, Pezet S. Characterisation of sensory abnormalities observed in an animal model of multiple sclerosis: A behavioural and pharmacological study. *Eur J Pain*. 2011;15(3):231.e1-231.e16. doi: <https://doi.org/10.1016/j.ejpain.2010.07.010>
36. Aicher SA, Silverman MB, Winkler CW, Bebo BF. Hyperalgesia in an animal model of multiple sclerosis: *Pain*. 2004;110(3):560-570. doi: <https://doi.org/10.1016/j.pain.2004.03.025>
37. Sadovnick AD, Remick RA, Allen J, et al. Depression and multiple sclerosis. *Neurol*. 1996;46(3):628-632. doi: <https://doi.org/10.1212/WNL.46.3.628>

38. Pollak Y, Orion E, Goshen I, Ovadia H, Yirmiya R. Experimental autoimmune encephalomyelitis-associated behavioral syndrome as a model of 'depression due to multiple sclerosis'. *Brain Behav Immun*. 2002;16(5):533-543. doi: [https://doi.org/10.1016/S0889-1591\(02\)00010-7](https://doi.org/10.1016/S0889-1591(02)00010-7)
39. Rodrigues DH, Vilela M de C, Lacerda-Queiroz N, et al. Behavioral investigation of mice with experimental autoimmune encephalomyelitis. *Arq Neuro psiquiatr*. 2011;69(6):938-942. doi: <https://doi.org/10.1590/S0004-282X2011000700018>
40. Potter LE, Paylor JW, Suh JS, et al. Altered excitatory-inhibitory balance within somatosensory cortex is associated with enhanced plasticity and pain sensitivity in a mouse model of multiple sclerosis. *J Neuroinflammation*. 2016;13(1):142. doi: <https://doi.org/10.1186/s12974-016-0609-4>
41. Buddeberg BS, Kerschensteiner M, Merkler D, Stadelmann C, Schwab ME. Behavioral testing strategies in a localized animal model of multiple sclerosis. *J Neuroimmunol*. 2004;153(1-2):158-170. doi: <https://doi.org/10.1016/j.jneuroim.2004.05.006>
42. Olechowski CJ, Tenorio G, Sauve Y, Kerr BJ. Changes in nociceptive sensitivity and object recognition in experimental autoimmune encephalomyelitis (EAE). *Exp Neurol*. 2013;241:113-121. doi: <https://doi.org/10.1016/j.expneurol.2012.12.012>
43. Rivat C, Becker C, Blugeot A, et al. Chronic stress induces transient spinal neuroinflammation, triggering sensory hypersensitivity and long-lasting anxiety-induced hyperalgesia: *Pain*. 2010;150(2):358-368. doi: <https://doi.org/10.1016/j.pain.2010.05.031>
44. Wang S, Tian Y, Song L, et al. Exacerbated mechanical hyperalgesia in rats with genetically predisposed depressive behavior: Role of melatonin and NMDA receptors: *Pain*. 2012;153(12):2448-2457. doi: <https://doi.org/10.1016/j.pain.2012.08.016>
45. Butler RK, Finn DP. Stress-induced analgesia. *Prog Neurobiol*. 2009;88(3):184-202. doi: <https://doi.org/10.1016/j.pneurobio.2009.04.003>
46. Falconi-Sobrinho LL, Anjos-Garcia T dos, Elias-Filho DH, Coimbra NC. Unravelling cortico-hypothalamic pathways regulating unconditioned fear-induced antinociception and defensive behaviours. *Neuropharmacology*. 2017;113:367-385. doi: <https://doi.org/10.1016/j.neuropharm.2016.10.001>
47. Falconi-Sobrinho LL, Coimbra NC. The nitric oxide donor SIN-1-produced panic-like behaviour and fear-induced antinociception are modulated by NMDA receptors in the anterior hypothalamus. *J Psychopharmacol*. 2018;32(6):711-722. doi: <https://doi.org/10.1177/0269881118769061>
48. Li M-J, Liu L-Y, Chen L, Cai J, Wan Y, Xing G-G. Chronic stress exacerbates neuropathic pain via the integration of stress-affect-related information with nociceptive information in the central nucleus of the amygdala: *Pain*. 2017;158(4):717-739. doi: <https://doi.org/10.1097/j.pain.0000000000000827>
49. Blaustein JD, Ismail N. Enduring influence of pubertal stressors on behavioral response to hormones in female mice. *Horm Behav*. 2013;64(2):390-398. doi: <https://doi.org/10.1016/j.yhbeh.2013.01.015>

Copyright: © **Bettenson et al.** This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.