



# Resolvin D2 attenuates chronic pain-induced depression-like behavior in mice

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## Abstract

**Aim:** We previously demonstrated that intracerebroventricular injection of resolvin D2 (RvD2), a bioactive lipid mediator derived from docosahexaenoic acid, ameliorated depression-like behavior in lipopolysaccharide-induced and chronic mild stress-induced mouse models of depression. In the present study, we examined the antidepressant effect of RvD2 on chronic pain-induced depression-like behavior.

**Methods:** To prepare the neuropathic pain model, mice were subjected to surgery for unilateral spared nerve injury. Two weeks after surgery, the antidepressant effect of RvD2 was examined using the tail suspension test.

**Results:** Chronic pain significantly increased immobility time, and this depression-like behavior was attenuated by intracerebroventricular injection of RvD2 (10 ng). No effect of RvD2 on the locomotor activity was observed.

**Conclusion:** RvD2 produces an antidepressant effect in a murine model of chronic pain-induced depression and may be a promising lead for the development of novel antidepressants.

## KEYWORDS

antidepressant, n-3 polyunsaturated fatty acid, pain, resolvin

## 1 | INTRODUCTION

Major depressive disorder is one of the most common psychiatric illnesses, resulting in enormous personal and socioeconomic burdens.<sup>1</sup> Because the available monoamine-based antidepressants have major limitations, including a delayed onset of treatment response (weeks to months) and low efficacy (more than one-third of depressed patients fail to respond to two or more antidepressants and are characterized as having treatment-resistant depression),<sup>2,3</sup> there is an unmet medical need for more effective and rapid-acting antidepressants. We previously reported that intracerebroventricular (i.c.v.) injection of resolvin D2 (RvD2), a bioactive lipid mediator derived from docosahexaenoic acid, ameliorated lipopolysaccharide

(LPS)-induced and chronic mild stress (CMS)-induced depression-like behavior using the tail suspension test (TST).<sup>4,5</sup> Comorbidity of chronic pain and depression has long been recognized in the clinic,<sup>6,7</sup> and several preclinical studies have reported depression-like behaviors in animal models of chronic pain.<sup>8</sup> Therefore, in the present study, we examined the antidepressant effect of RvD2 on chronic pain-induced depression-like behavior.

## 2 | MATERIALS AND METHODS

Male BALB/c mice (n = 96, 6 weeks old; Japan SLC) were group-housed (four per cage) at a constant ambient temperature (23 ± 1°C)

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under a 12-hour light/dark cycle (lights on 07:00), with food and water available ad libitum. All procedures were performed with the approval of the Institutional Animal Care and Use Committee at Hokkaido University. RvD2 was obtained as a solution in 100% ethanol from Cayman Chemical and stored at  $-80^{\circ}\text{C}$ . Immediately before use, the solution was diluted with sterile phosphate-buffered saline to a final ethanol concentration of 2% while minimizing exposure to light.

Figure 1A shows a schematic diagram of the experiments. To prepare the neuropathic pain model, mice were subjected to surgery for unilateral spared nerve injury (SNI).<sup>9</sup> Mice (6 weeks old) were anesthetized with isoflurane (induction: 3.0%, maintenance: 1.5%–2.0%) or chloral hydrate (400 mg/kg, intraperitoneal). The SNI model was prepared via unilateral ligations (left side) of the

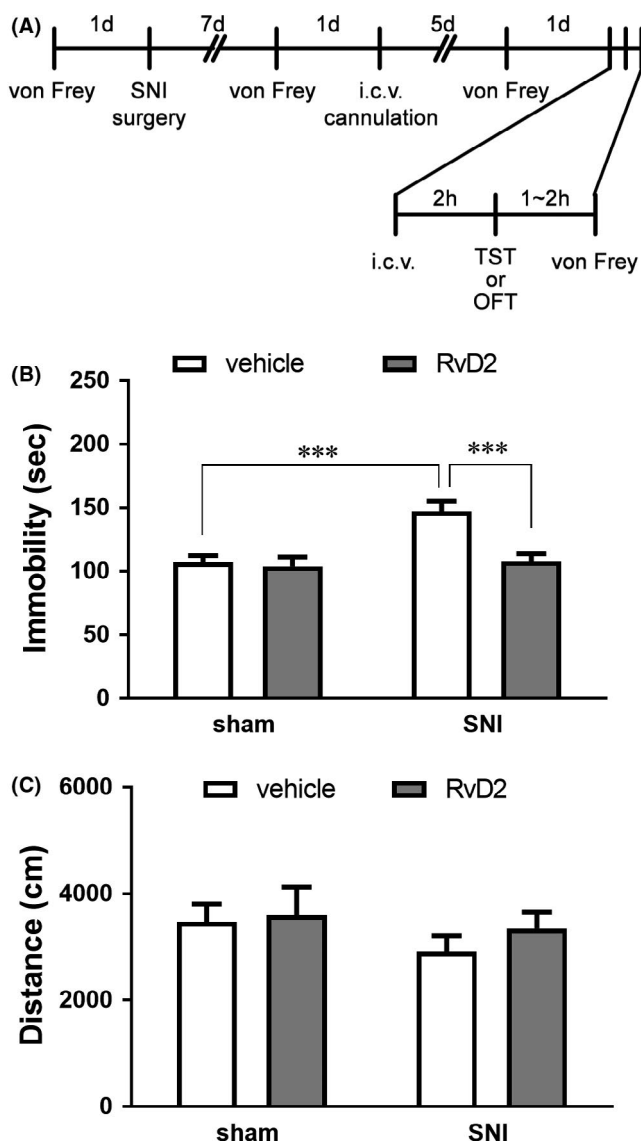
common peroneal nerve and tibial nerve using 5-0 silk, followed by cutting of the peripheral sides. Sham surgery included exposure of the sciatic nerve, but the common peroneal and tibial nerves were not injured. The von Frey test was conducted to measure thresholds to mechanical stimuli. Mice were placed on an elevated wire grid for at least 20 minute before testing. Pressure was applied to the plantar surface of the hind paw using von Frey filaments of different thicknesses (0.04, 0.07, 0.16, 0.40, 0.60, 1.00, and 2.00 g strength, starting at 0.16 g filament). The 50% paw withdrawal threshold was determined using the up-down method.<sup>10</sup> In case where continuous responses or nonresponses were observed to the exhaustion of the stimulus set, values of 0.04 g or 2.00 g were assigned, respectively.

Injection (i.c.v.) was performed as described previously.<sup>4</sup> Briefly, a guide cannula was implanted unilaterally above the lateral ventricle. Mice were injected with RvD2 (10 ng/5  $\mu\text{L}$ ) or vehicle (5  $\mu\text{L}$ ) at a rate of 2.5  $\mu\text{L}/\text{min}$  using an injection cannula. The TST was performed 2 hours after the injection to examine the antidepressant effect of RvD2 on chronic pain-induced depression-like behavior. Because we had previously reported that LPS- and CMS-induced depression-like behaviors were ameliorated 2 hours after i.c.v. injection of 10 ng of RvD2,<sup>4,5</sup> we employed the same dose and time schedule in this study. We previously showed the sustained (>24 hours) antidepressant effect of RvD2 in the CMS model.<sup>5</sup> Therefore, to reduce the number of animals used in this study, the von Frey test was performed 1–2 hours after the TST or open field test (OFT), using the same animals used for the TST/OFT. The duration of immobility was measured automatically for 6 minutes using an activity monitoring apparatus equipped with an infrared detector (SUPERMEX with CompACT FSS software; Muromachi Kikai). Mice that climbed their tail during the test period were excluded from the analyses ( $n = 8$ ). Locomotor activity was examined 2 hours after the injection of RvD2 using the OFT as described previously.<sup>4</sup> The total distance traveled was monitored for 10 minute using the EthoVision video-tracking system (Noldus Information Technology). Different cohorts of mice were used for the TST and OFT.

Histological analysis was performed after the behavioral tests as described previously.<sup>4</sup> Mice with incorrect injection ( $n = 20$ ) were excluded from analysis. Data are expressed as the mean  $\pm$  standard error of the mean. The data of the von Frey test were analyzed by Kruskal-Wallis test followed by Dunn's multiple comparison test. The data of the TST and OFT were analyzed by two-way ANOVA followed by the Holm-Sidak's *post hoc* test using GraphPad Prism 7 software (GraphPad Software).  $P < .05$  was considered statistically significant.

### 3 | RESULTS

Before RvD2 injection (2 weeks after the SNI or sham surgery), the pain threshold was significantly lower in the SNI group than the sham group (detailed data are shown in Supporting Information). There was no significant difference in the pain threshold between the sham-vehicle and sham-RvD2 groups or between the SNI-vehicle



**FIGURE 1** A, Schematic diagram of the experiments. B, Effect of RvD2 on immobility time in the TST. C, Effect of RvD2 on locomotor activity in the OFT. Data are expressed as the mean  $\pm$  standard error of the mean. \*\*\*  $P < .001$  (two-way ANOVA followed by the Holm-Sidak's *post hoc* test)



and SNI-RvD2 groups at 3–4 hours after RvD2 injection (detailed data are shown in Supporting Information). Chronic pain significantly increased the immobility time in the TST. RvD2 injection significantly attenuated this increased immobility time (Figure 1B; surgery:  $F_{1,33} = 11.20$ ,  $P = .0021$ ; treatment:  $F_{1,33} = 10.21$ ,  $P = .0031$ ; interaction:  $F_{1,33} = 7.431$ ,  $P = .0102$ ;  $n = 9–10$ ) but had no effect on immobility time in the sham group. Neither SNI surgery nor i.c.v. injection of RvD2 affected locomotor activity (Figure 1C; surgery:  $F_{1,25} = 1.155$ ,  $P = .2929$ ; treatment:  $F_{1,25} = 0.5705$ ,  $P = .4571$ ; interaction:  $F_{1,25} = 0.1653$ ,  $P = .6878$ ,  $n = 6–7$ ), indicating that the differences observed in the TST were not due to alterations in locomotor activity.

## 4 | DISCUSSION

We previously demonstrated that i.c.v. injection of RvD2 ameliorated depression-like behavior in LPS- and CMS-induced mouse models of depression.<sup>4,5</sup> The present study using a murine neuropathic pain model extends those findings by showing that i.c.v. injection of RvD2 reversed chronic pain-induced depression-like behavior. RvD2 may be a promising lead for the development of novel antidepressants.

In our previous study using an LPS-induced mouse model of depression, the antidepressant effect of RvD2 was blocked by pretreatment with rapamycin, an inhibitor of mechanistic target of rapamycin complex 1 (mTORC1), suggesting the involvement of mTORC1 signaling,<sup>4</sup> like rapid-acting antidepressants such as ketamine and scopolamine.<sup>11–13</sup> Thus, the antidepressant effect of RvD2 on chronic pain-induced depression-like behavior observed in this study may be mediated by mTORC1 activation. Alternatively, growing evidence demonstrates that inflammatory responses are involved in the pathophysiology of depression<sup>14,15</sup> and that microglia become activated in various brain regions in animal models of neuropathic pain.<sup>16</sup> RvD2 is a specialized proresolving lipid mediator, producing potent anti-inflammatory and proresolving actions in various animal models of inflammation.<sup>17</sup> For instance, Tian et al. reported that RvD2 suppressed LPS-induced mRNA expression of pro-inflammatory cytokines in primary microglial cells.<sup>18</sup> Thus, RvD2 may attenuate chronic pain-induced depression-like behavior via anti-inflammatory and proresolving actions. Further studies are needed to clarify the underlying mechanisms of the antidepressant effect of RvD2.

Intrathecal injection of RvD2 (10 ng) suppressed heat hyperalgesia induced by plantar injection of complete Freund's adjuvant.<sup>19</sup> Klein et al. reported that intrathecal administration of RvD2 (40 ng) suppressed mechanical allodynia in a fibromyalgia-like model induced by reserpine in mice and that intravenously administered RvD2 also suppressed mechanical allodynia, suggesting that systemically administered RvD2 may be transferred to the central nervous system and exert its effects there.<sup>20</sup> However, a much higher dose (300 ng) was required for intravenous administration to produce an anti-allodynic effect than intrathecal administration. To our knowledge, there are no reports on the anti-allodynic effect of

i.c.v. administration of RvD2. In this study, the anti-allodynic effect of i.c.v. administered RvD2 was not observed. However, this study has limitations that most of the animals in the SNI group showed withdrawal responses to the thinnest von Frey filament used in this study (0.04 g) and that the von Frey test was conducted only at one time point after the injection of RvD2. Further studies with von Frey test using thinner filaments (0.02 g and 0.008 g) at multiple time points after RvD2 injection are needed for detailed analysis of the anti-allodynic effect of i.c.v. administered RvD2.

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## CONFLICT OF INTEREST

The authors declare no conflict of interests.

## AUTHOR CONTRIBUTIONS

MM and SD conceptualized the studies. HS performed experiments and analyzed data. H.-IN and MM analyzed data and wrote the paper with editing contributions from all authors.

## ANIMAL STUDIES

All animal experiments were approved by the Institutional Animal Care and Use Committee at Hokkaido University.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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