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Transplant-mediated enhancement of spinal cord GABAergic inhibition reverses paclitaxel-induced mechanical and heat hypersensitivity

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Abstract

Decreased spinal cord GABAergic inhibition is a major contributor to the persistent neuropathic pain that can follow peripheral nerve injury. Recently, we reported that restoring spinal cord GABAergic signaling by intraspinal transplantation of cortical precursors of GABAergic interneurons from the embryonic medial ganglionic eminence (MGE) can reverse the mechanical hypersensitivity (allodynia) that characterizes a neuropathic pain model in the mouse. Here we show that MGE cell transplants are also effective against both the mechanical allodynia and the heat hyperalgesia produced in a paclitaxel-induced, chemotherapy model of neuropathic pain. To test the necessity of GABA release by the transplants, we also studied the utility of transplanting MGE cells from mice with a deletion of VGAT, the vesicular GABA transporter. Transplants from these mice, in which GABA is synthesized but cannot be stored or released, had no effect on mechanical hypersensitivity or heat hyperalgesia in the paclitaxel model. Taken together, these results demonstrate the therapeutic potential of GABAergic precursor cell transplantation in diverse neuropathic pain models and support our contention that restoration of inhibitory controls through release of GABA from the transplants is their mode of action.

INTRODUCTION

GABAergic and glycinergic inhibitory controls in the spinal cord dorsal horn are major contributors to the regulation of pain messages from the periphery to the brain [3,4,48]. Following peripheral nerve injury, however, a profound loss of these inhibitory controls can occur, contributing to increased spinal cord neuronal hyperexcitability. Whether there is frank loss of inhibitory interneurons following nerve injury is unclear [34,38,41], however electrophysiological and biochemical analyses [26,9,16,18,21] unquestionably demonstrated a profound loss of GABAergic inhibition. The behavioral manifestation of these changes is ongoing pain, and depending on the particular nerve injury, a persistent mechanical and thermal (heat and/or cold) hypersensitivity, hallmarks of neuropathic pain.

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Traditional treatment modalities for neuropathic pain involve pharmacological suppression of the neuronal hyperexcitability. Not surprisingly, therefore, the most common therapies for neuropathic pain rely on anti-convulsants with proven efficacy in the management of epilepsy, another condition associated with hyperexcitability. An alternative is to take a disease-modifying therapeutic approach, namely one that restores inhibitory tone by rewiring inhibitory circuits in the spinal cord [22]. Following upon the studies of Baraban et al [2] in a mouse model of epilepsy, w demonstrated that intraspinal transplantation of precursors of cortical GABAergic interneurons derived from the mouse medial ganglionic eminence (MGE) can completely reverse the mechanical hypersensitivity produced in a peripheral nerve injury model of neuropathic pain [7]. The MGE approach is also very effective at reducing the exacerbated scratching [6] caused by loss of GABAergic spinal cord interneurons in a mouse model of chronic itch. On the other hand, the MGE transplants were not effective in the formalin model of postoperative/inflammatory pain. As the pain behaviors in both inflammatory and neuropathic pain models can be reduced by administering GABA agonists at the level of the spinal cord [24,37,20,25,27,30], we concluded that the MGE cells did not act as therapeutic pumps that provide a continuous release of GABA, but rather served to repair the GABAergic circuitry that was altered by the nerve injury.

In the present report, we show that the MGE cell transplant is also effective in a neuropathic pain condition in which the levels of glutamic acid decarboxylase (GAD), the enzyme required for GABA synthesis, are not reduced. Specifically, an MGE cell transplant reversed both the mechanical and heat hypersensitivity produced in a chemotherapy (paclitaxel)-induced model of neuropathic pain [40,14]. Finally, to test the necessity of GABA release from the transplants, we studied the utility of transplanting MGE cells from mice with a deletion of VGAT, the vesicular GABA transporter. We report that transplants from these mice, in which GABA is synthesized but cannot be stored or released, had no effect on the mechanical hypersensitivity or heat hyperalgesia in the paclitaxel model. We conclude that the anti-hyperalgesic effect of MGE transplants is indeed GABA-mediated.

METHODS

Mouse lines

All experiments were reviewed and approved by the Institutional Care and Animal Use Committee at the University of California San Francisco. MGE cells were dissected from transgenic mice that express GFP under the control of the promoter of the GAD67 enzyme [$Gad1^{tm1.1Tama}$; 43]. All transplants were performed on C57BL/6 adult male mice (8 weeks old). The VGAT mutant mice were generated by crossing a floxed-VGAT mouse [44] with a mouse that expresses Cre under the control of the I12b enhancer [36]. Double transgenic I12b-fl.VGAT mice were then crossed to R26-Tdtomato (TdT) reporter mice [29] so that MGE cells dissected from the triple I12b-fl.VGAT-TdT transgenic mice could be detected after transplantation.

Paclitaxel-induced neuropathic pain model

To produce mechanical and heat hypersensitivity that mimics a chemotherapy-induced neuropathic pain conditions, we used the paclitaxel model [40,14]. Briefly, we injected adult wild-type mice with 1mg/kg of paclitaxel (Sigma), which was dissolved in 40% DMSO. Mice were injected intraperitoneally 4 times, every other day.

Transplantation of MGE cells

Tissue dissections and transplantation were performed as described previously [7]. Briefly, one week after the last injection of paclitaxel, 6 to 8 week old male mice were anesthetized with an intraperitoneal injection of ketamine (60 mg/kg)/xylazine (8 mg/kg). A dorsal hemilaminectomy was performed at the level of the lumbar enlargement to expose 2 segments (~1.5–2.0 mm) of lumbar spinal cord. The dura mater was incised and reflected. We loaded a cell suspension (1.0μl) containing $5x10^4$ MGE cells (dissected from the ventricular and subventricular layers of the anterior part of the MGE from E12.5 to E13.5 embryonic day GAD67-GFP or VGAT mutant transgenic mice) into a glass micropipette that was prefilled with mineral oil. The micropipette was connected to a microinjector mounted on a stereotactic apparatus. The cell suspension injections were targeted to the dorsal horn, ipsilateral to the nerve injury. We made 5–6 single injections over the two segments (for a total of 1.0 μl). Control groups were injected with an equivalent volume of Dulbecco's Modified Eagle Medium (DMEM). The wound was closed and the animals were allowed to recover after which they were returned to their home cages. Animals were killed at the end of the behavioral tests (4 weeks post-transplantation) for anatomical analyses.

Cell counts

Estimation of survival rates was performed as described previously [7]. Briefly, the percentage of surviving MGE cells was determined by counting all GFP+ cell bodies in 10 spinal cord sections (separated by 100 μm). The average number of GFP+ cells per section was then extrapolated to the total number of spinal cord sections that contained GFP+ cells, using the formula: Total GFP = A x B/2 (where A is the average number of GFP+ cells per section and B the total number of spinal cord sections containing GFP+ cells). Given the thickness of the spinal cord sections and the size of the MGE cells, we only included every other section so that cells were not counted twice. For these quantification studies, we used 5 animals per group.

Quantitative PCR

Quantitative assays were performed as described previously [6, 7]. Mice were transplanted with medium (n=3) or MGE cells (n=5) 1 week after the last injection of paclitaxel, and killed 2 weeks after transplantation. Naïve (uninjured) mice (n=3) were also used as controls. We rapidly dissected the lumbar spinal cord that contained the transplants (a comparable region was dissected from naïve animals) and divided the cord into ipsilateral and contralateral halves (containing both dorsal horn ventral tissue). We extracted mRNA from the spinal cord tissue using the RNeasy Minikit from Qiagen and then reverse-transcribed 200 ng of purified mRNA into cDNA using Superscript III (Invitrogen). The mRNA levels for GAD65, GAD67 and β-actin were quantified with a Realplex^2 real-time

PCR system (Eppendorf) using SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK). Ratios of GAD65 and GDA67 to β-actin mRNA were compared and analyzed by a two-way ANOVA.

Behavioral analyses

Mechanical sensitivity was assessed by placing animals on an elevated wire mesh grid and stimulating the hind paw with von Frey hairs and using the up-down paradigm [12] to define threshold. Heat thresholds were assessed using the Hargreaves' reflex test of heat pain sensitivity [8]. Animals were tested once per day, on 3 different days (every other day) before paclitaxel injections (to determine baseline) and once, 2 days after the last injection of paclitaxel (for hypersensitivity testing). Mechanical and heat thresholds were recorded 7, 14, 21 and 28 days after MGE/medium injections. The investigator performing the behavioral tests was blind to treatment (cell medium or MGE injection). One month after transplantation, the MGE transplanted animals were killed and the spinal cord was immunostained for the presence of GFP+ cells. For this series of experiments, all animals injected were *successfully* transplanted (defined as having at least 1 GFP+ cell per section) and their behavior was subsequently analyzed. Importantly, the investigator performing this anatomical analysis was not the investigator who performed the behavior analysis and thus was blind to the behavior results.

Statistical analyses

Data are expressed as mean +/− SEM (standard error of the mean), where n represents the number of mice tested. Raw data for the mechanical and heat withdrawal thresholds obtained in the course of the study were analyzed by a two-way ANOVA followed by a Tukey post hoc test. Asterisks (*) indicate statistically significant differences between groups, with * = $p < 0.05$; ** = $p < 0.01$ and *** = $p < 0.001$.

Antibodies and Immunohistochemistry

All experiments were performed as previously described [7]. We used the following antibodies: Rabbit anti-GFP (1:2000, Molecular Probe), chicken anti-GFP (1:2000, Abcam), rabbit anti-GABA (1:2000, Sigma), rabbit anti-ATF3 (1:2000; Santa Cruz), rabbit anti-Iba1 (1:1000; Wako) and mouse anti-NeuN (1:2000, Chemicon).

RESULTS

Transplanted MGE cells reverse the neuropathic pain phenotype induced by paclitaxel

We previously studied the utility of the transplant in a traumatic, nerve injury (SNI) model of neuropathic pain, which involves transection of two of the three branches of the sciatic nerve [39]. This model is characterized by profound mechanical hypersensitivity. As heat hypersensitivity is uncommon in the SNI model, we could not determine whether there is any modality specificity to the recovery produced by the transplants. To address this question, here we examined the consequences of transplanting MGE cells in a mouse model of chemotherapy (paclitaxel)-induced neuropathic pain. Paclitaxel produces a bilateral, long-lasting neuropathy in mice and rats [40,1,35], with both mechanical and heat hypersensitivity.

Figure 1 illustrates the profound reduction of mechanical and heat thresholds produced 2 days after the last injection of paclitaxel (−~60% and −~40% respectively). One week after the hypersensitivity developed, the mice received a suspension of MGE cells (transplanted group) or medium alone (no cells, control group) unilaterally into the lumbar spinal cord. Figures 1A–B show that in control animals the mechanical allodynia and heat hyperalgesia persisted throughout the 4 weeks of observation. In MGE-transplanted mice, however, we recorded a rapid and significant reduction of both paclitaxel-induced heat hyperalgesia and mechanical allodynia. Importantly, the reversal of the hypersensitivity was limited to the hindlimb ipsilateral to the transplant. The recovery of function was especially notable for the heat hyperalgesia; heat withdrawal thresholds returned to pre-injury baseline levels between the 1st and 2nd week post-transplantation. In contrast, we observed a more gradual reduction of the mechanical allodynia, with complete recovery only occurring (on average) by 3 weeks post-transplantation.

Survival of the MGE transplants in the spinal cord of paclitaxel-treated mice

Figure 2 illustrates a representative example of GFP+ transplanted MGE cells in the spinal cord of a paclitaxel-injected mouse. As we reported for transplants in mice that underwent traumatic nerve injury (SNI), we found that MGE cells survive well in paclitaxel-treated mice and distribute throughout the dorsal horn of the spinal cord. Transplanted cells extend long, ramified processes, restricted to the side of the transplant. In this series of experiments, we estimated that 5.8% ± 2.4 of MGE cells (from an initial injection of 50,000 cells) survived after transplant, which is slightly higher than what we observed in previous experiments. Whether the increased survival in the paclitaxel mice reflects improvement in our transplant protocol, or a differential effect of the nerve injury conditions on cell survival, is unclear. The survival rate is low compared to previous studies that transplanted MGE cells into the neonatal cortex but comparable to studies that transplanted MGE cells into the striatum. This suggests that the tissue in which the cells are transplanted may influence their survival. Furthermore, we do not believe that cell death post-transplantation is the major contributor to the limited recovery of transplanted cells [7]. Rather, and as described in our previous study, the survival rate may have been underestimated. We believe that a large number of cells are lost during the injection (e.g., cells leak out of the injection site; some cells are trapped in the pia and there is likely some cell death in the course of the injection).

Necessity of GABA release for the anti-hyperalgesic effects of MGE transplants

To determine whether MGE-derived GABA, in fact, mediates the reversal of the mechanical allodynia and heat hyperalgesia, in a different group of animals we transplanted cells isolated from the MGE of mice in which there is a deletion of the gene that encodes the vesicular GABA transporter (VGAT mutant). In these mice, MGE cells synthesize GABA but because GABA cannot be stored into vesicles, there is no release. To generate these mice, we crossed floxed VGAT mice (fl.VGAT; [44]) with mice that express Cre recombinase under the control of the I12b enhancer, which results in Cre expression selectively in forebrain inhibitory interneurons [36]. Next we crossed double transgenic fl.VGAT/I12bCre mice to TdTomato (TdT) reporter mice so that the MGE cells derived from the fl.VGAT/I12bCre/TdT triple transgenic mice can be readily identified after transplantation. Figure 3C demonstrates successful transplant of the VGAT mutant MGE

cells into the spinal cord of the paclitaxel-injected mice. There was no statistical difference in the survival rate of VGAT mutant MGE cells [5.3% ± 3.1] compared to MGE cells from wild type mice. Double labeling with antibodies against GABA confirmed that the cells differentiated into GABAergic interneurons (NeuN+ and GABA+; Fig. 3D–E).

Most importantly, survival and/or integration of MGE cells is clearly not sufficient to reverse the neuropathic pain condition. Thus, in contrast to wild-type MGE cells, VGAT mutant MGE cells did not influence the mechanical allodynia or heat hyperalgesia induced by paclitaxel (Figures 3A–B). In fact, the behavior of mice that received the VGAT mutant MGE cells was not statistically different from that in the control (medium) group. We conclude that GABA release from MGE cells (and presumably the subsequent binding of GABA to spinal cord GABA receptors) indeed underlies the beneficial effects of MGE transplants.

Effects of MGE transplants on GAD expression levels in paclitaxel-treated mice

As others have reported, ipsilateral to the peripheral nerve injury we documented a significant decrease of the spinal cord expression levels of glutamic acid decarboxylase (GAD65 and 67), the biosynthetic enzyme for GABA. The decrease is presumed to contribute to the loss of inhibitory controls that characterize the neuropathic pain condition in this traumatic pain model. Consistent with our hypothesis that the MGE cells reestablish GABAergic tone, we found that the transplants normalized GAD mRNA levels. Interestingly, the GAD mRNA levels never exceeded those of normal mice. Here we used qPCR to determine whether comparable changes occur after paclitaxel-induced peripheral neuropathy. Somewhat surprisingly, compared to control mice we found no change in the mRNA spinal cord levels of GAD65 or 67 in the paclitaxel-injected mice (Fig. 2D). And as we observed previously, the transplants did not increase GAD mRNA levels above those in control mice. This latter result corroborates our hypothesis that the MGE transplants recapitulate endogenous spinal cord circuitry, rather than act as pharmacological pumps, which might be concluded if transplants in these animals increased GAD levels above normal.

Lack of ATF3 induction and Iba-1 upregulation after paclitaxel

The paclitaxel mouse model is thought to mimic a chemotherapy-induced neuropathic pain model. The lack of change in the spinal cord expression levels of GAD mRNA in the paclitaxel-injected animals (compared to control, naïve mice; Fig.2D) suggests that the mechanisms underlying the paclitaxel-induced hypersensitivity differ from those underlying neuropathic pain conditions that develop following nerve injury, at least at the doses used in the current study. As dorsal root ganglion (DRG) induction of ATF3 is a very sensitive marker of sensory neuron damage [5,46], we first asked whether ATF3 is induced in the DRG of paclitaxel-treated mice. We also immunostained the spinal cord of paclitaxel- and control (DMSO-injected) mice for Iba-1, a marker of activated microglia. Figure 4 shows that the number of ATF3-positive DRG neurons recorded one week after the last paclitaxel injection did not differ from that produced by the DMSO vehicle (Fig. 4A–B). The number of ATF3 positive neurons observed is also substantially lower than the number that we previously observed in mice that underwent peripheral nerve injury [5]. We also found

comparable levels of Iba-1 expression in the spinal cord of the paclitaxel and control mice (Fig. 4C–D). Again peripheral nerve injury produced a much more dramatic activation of microglia [42,47,45,31]. Based on these findings, we conclude that low doses of paclitaxel produce mechanical and heat hypersensitivity without inducing ATF3 or activating spinal cord microglia, at least at early time points (7 days).

DISCUSSION

We recently reported that MGE cells can completely reverse the mechanical hypersensitivity produced in a mouse model of peripheral nerve injury-induced neuropathic pain [7]. We also demonstrated that the MGE cells have anti-pruritic effects in a Bhlhb5 mutant mouse model of neuropathic chronic itch [6]. Because a profound deficit in spinal cord GABA signaling is a major contributor to the exacerbated pain and itch that characterize these animal models, there was a strong rationale for considering this therapeutic approach. In the present study, however, we found that the MGE transplant approach has beneficial effects even in a pathological condition in which a major indicator of GABAergic functionality, namely the level of GAD enzyme, is intact. Our results suggest that transplant-mediated enhancement of GABAergic tone may be a promising therapeutic approach to treat a wide variety of neuropathic pain and itch conditions, with varying etiologies.

Although it is likely that there is some commonality in the mechanisms underlying the hyperalgesia induced by paclitaxel and other peripheral neuropathies (e.g., increased spontaneous activity and increased responsiveness of dorsal horn nociresponsive neurons [10], selective changes produced in the paclitaxel model may be more sensitive to the inhibitory action of the transplant. Most importantly, as GAD mRNA levels are unchanged in the paclitaxel model, at least when low doses are used, any hypersensitivity resulting from loss of inhibitory function likely arises from alterations in GABAergic inhibitory circuitry. Indeed, in paclitaxel-injected mice, Chen et al. [13] recently reported impaired spinal cord GABAergic synaptic inhibition due to a chloride gradient imbalance. On the other hand and consistent with the results of Flatters et al. [17], we did not find significant ATF3 induction in DRG cells after paclitaxel. We also did not observe the microglial activation that is typically linked to nerve damage and ATF3 induction. We presume that the reports of ATF3 induction in some studies reflected the very high doses of paclitaxel that were administered [23,32,28, 33]. In other words, the molecular and cellular changes generated by paclitaxel are likely dose-dependent.

In contrast to peripheral nerve injury models in which a unilateral mechanical allodynia develops ipsilateral to the injury, paclitaxel injections generate a long lasting, bilateral (i.e., systemic) mechanical and heat hypersensitivity [40,14]. The bilaterality of the clinical symptoms provided an opportunity to assess the topographic nature of the anti-nociceptive effects of the transplants. In fact, we recorded a significant increase of both mechanical and heat thresholds in the MGE transplanted animals, which was limited to the hindlimb ipsilateral to the transplant side. This finding is consistent with our general observation that there is no contralateral migration of the transplanted cells. The fact that the transplants are effective against different modalities of pain, which are generated by distinct spinal circuits [11,49], also supports our contention that the transplants integrate in a random manner. In

fact, MGE cells extend long and ramified processes throughout the spinal cord and likely establish connections with a wide variety of spinal cord neurons, including those that respond to algogenic and others that respond to pruritic stimuli. In this respect, the inhibitory action of MGE transplants mimics the broad inhibitory effects that GABA agonists exert in different pain models [19,15,50]. On the other hand, as the transplants appear to normalize, rather than enhance GABA functionality, MGE transplants may not produce the side effects that are often associated with systemic or intrathecal administration of GABAergic agents.

Is GABA release, in fact, necessary for the antihyperalgesic effects of the transplants? Based on the neurochemical characterization of the transplanted neurons (i.e. they express GABA) and the fact that the transplants normalized levels of the GAD enzyme, which decrease after nerve injury, we previously hypothesized that MGE-derived GABA indeed mediates the anti-hyperalgesic effects of the transplants. Here we provide strong evidence in support of this hypothesis. Thus, transplants of MGE cells that can no longer release GABA (derived from VGAT mutant mice) were completely ineffective in the paclitaxel-injected mice. Although we did not directly assess the spinal cord integration of the VGAT mutant MGE cells, their comparable survival rate and differentiation into GABAergic interneurons suggest that the absence of VGAT did not negatively impact the ability of these transplants to integrate into the spinal cord. Rather, we consider that the inability of the cells to release GABA is the main contributor to the lack of efficacy of the mutant cells against paclitaxel-induced hypersensitivity. It is, of course, possible that other properties of the transplanted cells (e.g., release of other neurotransmitters and neuromodulators) contribute to the anti-hyperalgesic effect of the transplant, however release of GABA appears to be essential.

Is the spinal cord integration of the transplants critical to the ability to exert an antinociceptive action? Although we demonstrated in our previous study that integration of the cells into host circuits occurs and presumed that this integration was required for function, the present study suggests that it is possible that some more generalized release of GABA could also have beneficial effects. As the MGE cells are GFP+ at the time of transplant, we can conclude that the GAD promoter, which drives the GFP expression, is functional and thus the MGE cells have the ability to synthesize GABA soon after transplant. The fact that we observed recovery within one week after transplant, even before the neuronal phenotype of the transplant is established [7], suggests, therefore, that there is a very early contribution of GABA release. However, we do not believe that the MGE cells are functioning as therapeutic pumps that provide a continuous release of GABA. Rather the pronounced integration that we have demonstrated suggested that the transplants re-establish some critical component of the GABAergic circuitry that was altered by the nerve injury. Consistent with this conclusion we previously demonstrated that MGE transplants are not effective in the formalin test [7], a model of postoperative/inflammatory pain that responds well to intrathecal administration of GABA agonists [24,37,20,25,27,30].

In conclusion, in the present study we provide further evidence for the broad therapeutic potential of MGE cell transplantation. In addition to counteracting the neuropathic pain phenotype following traumatic nerve damage, the transplants are remarkably effective against both mechanical and heat hyperalgesia in a chemotherapy-induced model of neuropathic pain. Importantly, we also demonstrate that the utility of the transplants is not

limited to conditions in which there is a profound loss of GABAergic inhibitory controls pain. Anesthesiology 2000;92:876–80.

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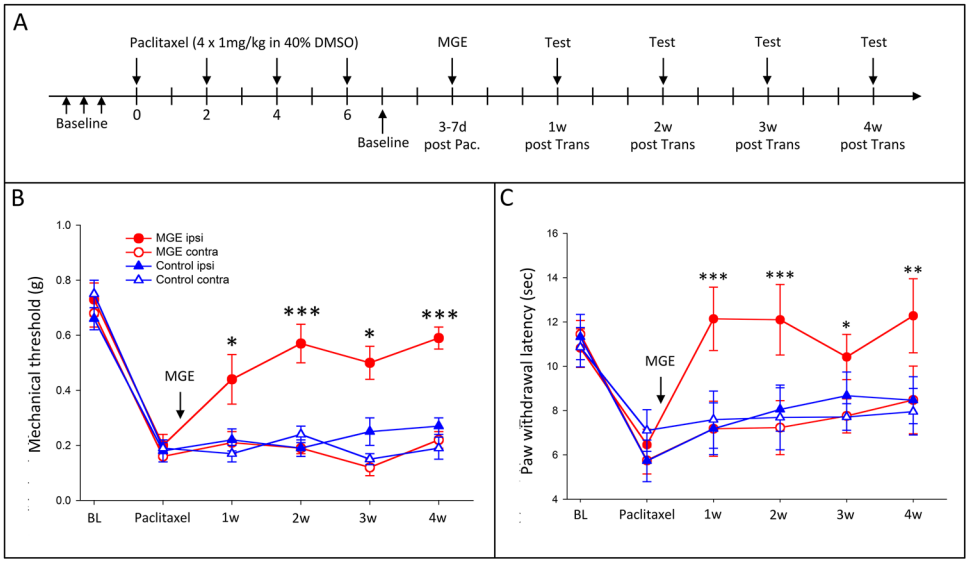

Figure 1. MGE transplants reverse both mechanical allodynia and heat hyperalgesia in the paclitaxel (chemotherapy)-induced neuropathic pain model

(A) Time line of Paclitaxel (Pac) injections, MGE transplantation (Trans) and behavioral testing. (B–C) Paclitaxel induces a long-lasting and bilateral mechanical allodynia and heat hyperalgesia. MGE cells normalized both mechanical (B) and heat (C) thresholds ipsilateral to the transplant, without altering thresholds of the contralateral hindpaw.

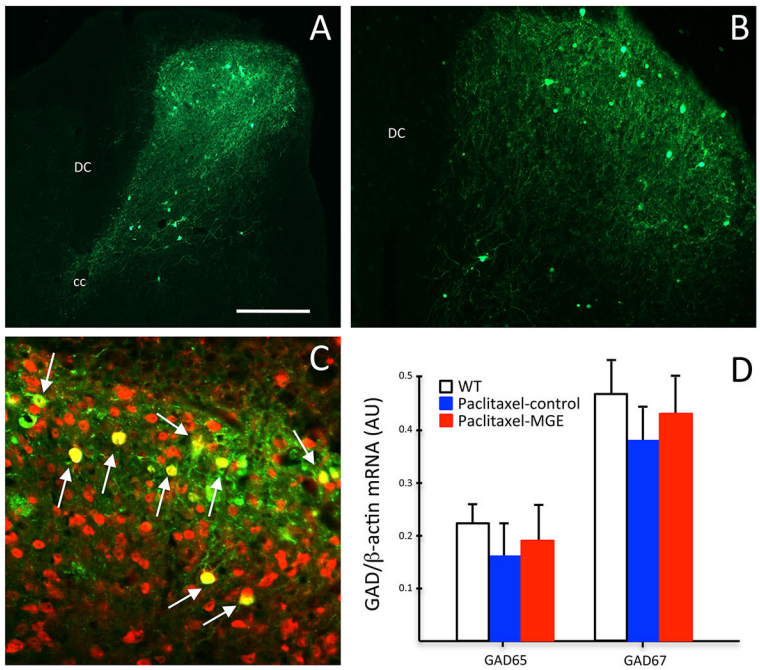

Figure 2. Survival of MGE transplants in the spinal cord of Paclitaxel-injected mice

(A–B) GFP-expressing MGE transplants survived and dispersed throughout the dorsal horn. Transplanted cells did not migrate to the contralateral side of the cord. (C) Most GFP+ MGE cells expressed NeuN, a marker of mature neurons. DC: dorsal columns; CC: central canal. Scale bar equals 150 μm in A, 75 μm in B and 50 μm in B–C. (D) In contrast to peripheral nerve injury [7], Paclitaxel did not alter the spinal cord mRNA levels of the GAD65 and 67 biosynthetic enzymes and the mRNA levels were not affected by the transplants. AU: arbitrary units.

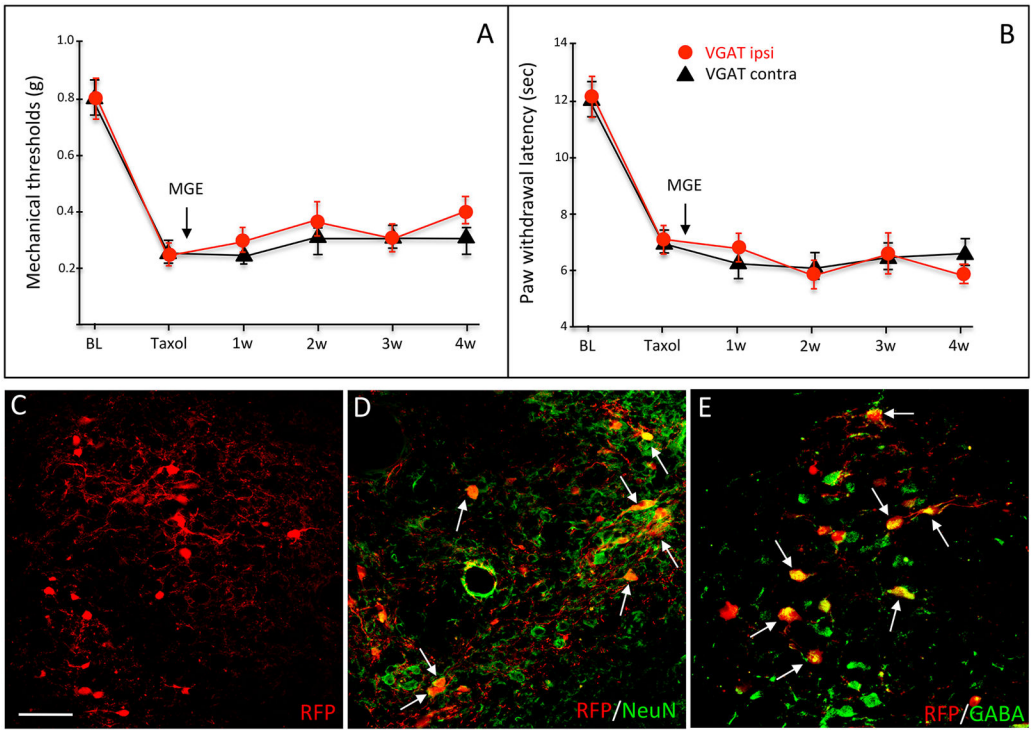

Figure 3. Anti-hyperalgesic effects of MGE transplants require GABA

(A–B) Transplants of MGE cells from VGAT mutant mice, which can neither store nor release GABA are ineffective against mechanical (A) and heat (B) hypersensitivity induced by Paclitaxel. (C–E) Transplanted MGE cells from the VGAT mutant mice that express a TdTomato reporter survive in lumbar spinal cord (red, C). Similar to wild-type MGE cells, transplanted VGAT mutant MGE cells express NeuN (green, D) and GABA (green, E), demonstrating that they differentiated into GABAergic interneurons. Arrows in D and E point to examples of double-labeled MGE cells. Scale bar equals 50 μm.

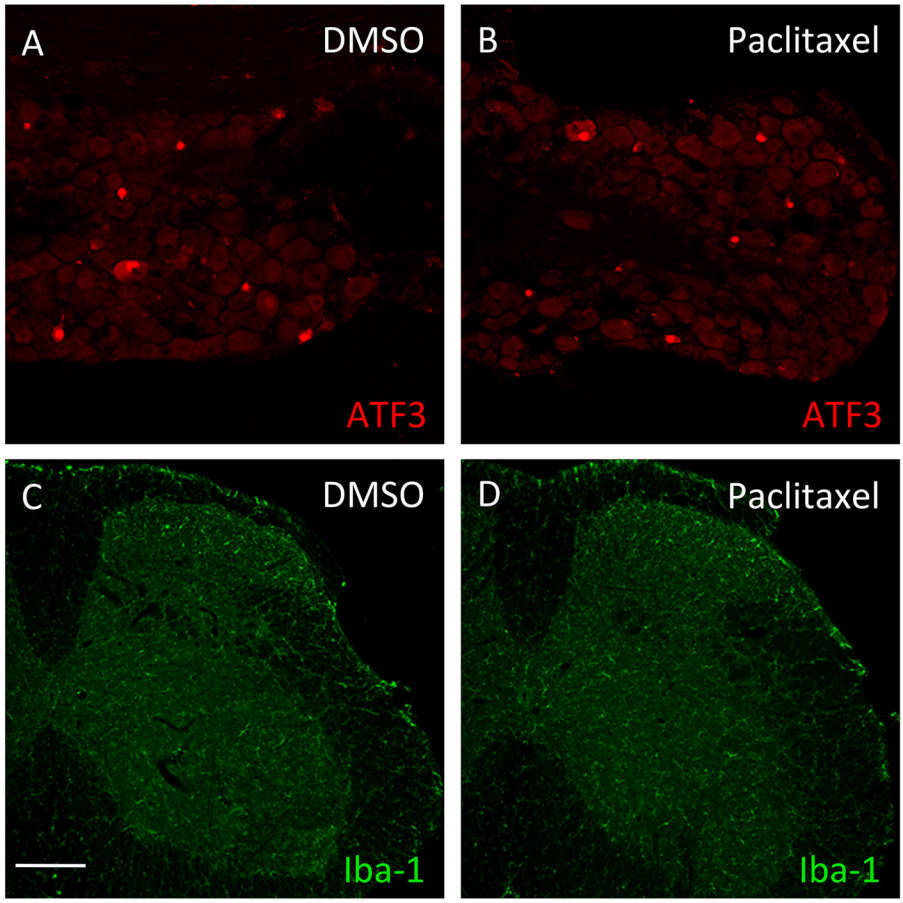

Figure 4. Paclitaxel induces hypersensitivity via a process that differs from that produced by peripheral nerve injury

Systemic injections of vehicle (40% DMSO, A) or Paclitaxel (B) induce low and comparable expression levels of ATF3 (red) in lumbar DRG neurons (A–B). There is also minimal lumbar spinal cord upregulation of Iba-1 (C–D), a marker of microglia, and again the patterns in the DMSO and Paclitaxel-treated mice did not differ. Scale bar equals 100 μm in A–B and 200 μm in C–D.