

# A simple and fast method to image calcium activity of neurons from intact dorsal root ganglia using fluorescent chemical $\text{Ca}^{2+}$ indicators

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## Abstract

Chemical calcium indicators have been commonly used to monitor calcium ( $\text{Ca}^{2+}$ ) activity in cell bodies, i.e., somata, of isolated dorsal root ganglion neurons. Recent studies have shown that dorsal root ganglion somata play an essential role in soma–glia interactions and actively participate in the transmission of nociceptive signals. It is therefore desirable to develop methods to study  $\text{Ca}^{2+}$  activity in neurons and glia in intact dorsal root ganglia. In our previous studies, we found that incubation of intact dorsal root ganglia with acetoxymethyl dye resulted in efficient  $\text{Ca}^{2+}$  dye loading into glial cells but limited dye loading into neurons. Here, we introduce a useful method to load  $\text{Ca}^{2+}$  dyes in intact dorsal root ganglion neurons through electroporation. We found that electroporation greatly facilitated loading of Fluo-4 acetoxymethyl, Oregon green bapta-1-488 acetoxymethyl, and Fluo-4 pentapotassium salt into dorsal root ganglion neurons. In contrast, electroporation did not further facilitate dye loading into glia. Using electroporation followed by incubation of acetoxymethyl form  $\text{Ca}^{2+}$  dye, we can load acetoxymethyl  $\text{Ca}^{2+}$  dye well in both neurons and glia. With this approach, we found that inflammation induced by complete Freund's adjuvant significantly increased the incidence of neuron–glia interactions in dorsal root ganglia. We also confirmed the actions of capsaicin and morphine on  $\text{Ca}^{2+}$  responses in dorsal root ganglion neurons. Thus, by promoting the loading of  $\text{Ca}^{2+}$  dye in neurons and glia through electroporation and incubation,  $\text{Ca}^{2+}$  activities in neurons and neuron–glia interactions can be well studied in intact dorsal root ganglia.

## Keywords

Electroporation, dorsal root ganglion, calcium, calcium dye, inflammation, pain, neuron–glia interaction

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## Introduction

Isolated and round-shaped cell bodies (somata) of dorsal root ganglion (DRG) neurons have been used traditionally as a model cell preparation to study electric properties of central and peripheral sensory terminals based on the assumption that the somata and terminals have similar electrical characteristics<sup>1</sup> and somata are not actively participating in sensory signaling.<sup>2</sup> This view has recently been challenged.<sup>3–7</sup> We and others have shown that DRG somata are essential in soma–glial interactions and actively participate in the transmission of nociceptive signals.<sup>5,6,8–12</sup>

To better understand the involvement of neuronal somata and the role of soma–glia interactions in pain signaling, some recorded the activity of DRG somata

using sharp electrodes and/or patch electrodes and studied changes in  $\text{Ca}^{2+}$  signaling through the injection of a salt form of fluorescent  $\text{Ca}^{2+}$  dye into a small number of neurons in intact DRGs.<sup>8,9,13–17</sup> We bulk loaded Fluo-4 acetoxymethyl  $\text{Ca}^{2+}$  dye in L4 or L5 ganglia with sciatic nerves attached.<sup>5,6</sup> The approach allowed us to examine neuron–glia interactions in DRGs under different experimental conditions. This dye loading technique resulted in

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efficient  $\text{Ca}^{2+}$  dye loading into glial cells. However, only a limited number of neurons were loaded. We therefore tested various  $\text{Ca}^{2+}$  dye loading techniques and found that  $\text{Ca}^{2+}$  dyes could be efficiently loaded in intact DRG neurons through electroporation. Conditions to optimize the study of neuron–glia interactions were explored further.

## Materials and methods

### Animals

Male adult (100–250 g) Sprague Dawley rats were used in this study. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Texas Medical Branch and were in accordance with the guidelines of the National Institutes of Health and of the International Association for the Study of Pain.

### Complete Freund's adjuvant induced inflammation

To induce inflammation, animals were anesthetized with isoflurane (5%), and complete Freund's adjuvant (CFA; 50  $\mu\text{l}$ ) was injected into the plantar surface of the left hindpaw. Solution of CFA was prepared by mixing *Mycobacterium butyricum* (10 mg/ml; Difco, Detroit, MI) in a peanut oil–saline (1:1) emulsion. Signs of localized inflammation in left hindpaw, such as redness and swelling, were seen several hours later.  $\text{Ca}^{2+}$  imaging experiments were performed three to nine days after the CFA injection.

### Retrograde loading of dextran form of $\text{Ca}^{2+}$ dye

In some rats, under isoflurane anesthesia (initiated at 5% and maintained at 2%), the left sciatic nerve was transected in mid thigh, and the distal cut end of the nerve was tightly ligated with 5-0 silk. The proximal cut end was soaked in  $\sim 10 \mu\text{l}$ , 1% calcium green-1 dextran 3000 (Ca green dextran) solution for 30 min. The incisions were closed in layers. Animals were returned to their cages under standard colony conditions, with food and water available *ad libitum*. On day 5 after the  $\text{Ca}^{2+}$  dye loading, the L4 and L5 DRGs were taken out to prepare intact DRG preparations for  $\text{Ca}^{2+}$  imaging experiments as described below.

### Intact DRG preparation

Under pentobarbital (50 mg/kg, i.p.) anesthesia, L4 or L5 DRGs were removed from normal (including those that had undergone retrograde  $\text{Ca}^{2+}$  dye loading) and CFA rats, in some cases, with the sciatic nerve attached. After carefully removing the capsule, DRGs were recovered in

an extracellular solution for 30 min at room temperature. The extracellular solution contained (in mM) 115 NaCl, 5.6 KCl, 1  $\text{NaH}_2\text{PO}_4$ , 2.0  $\text{CaCl}_2$ , 1  $\text{MgCl}_2$ , 11 glucose, and 25  $\text{NaHCO}_3$  and was bubbled with  $\text{O}_2$ .

### Chemical agents

All  $\text{Ca}^{2+}$  dyes were purchased from Thermo Fisher Scientific (Waltham, MA). Morphine was obtained from Hospira (Lake Forest, IL) and capsaicin was from Sigma (St. Louis, MO).

### Calcium imaging

Calcium imaging studies were conducted on intact DRG preparations at room temperature. Ganglia were electroporated with the Amaxa Neucleofector II. Briefly, ganglia with or without attached sciatic nerves were put into a cuvette which contained 100  $\mu\text{l}$  of extracellular solution with  $\text{Ca}^{2+}$  dye for electroporation. The protocols used in the study were A003, C002, or C003. Since these three electroporation protocols did not yield significant differences in  $\text{Ca}^{2+}$  dye loadings, results were combined in our data analyses. For acetoxymethyl (AM)-form  $\text{Ca}^{2+}$  dyes, ganglia were incubated with the same  $\text{Ca}^{2+}$  dye solution for 1 h immediately after electroporation unless stated otherwise.

The concentrations of  $\text{Ca}^{2+}$  dyes used were as follows: Fluo-4 AM (0.92 mM) and Oregon Green<sup>TM</sup> 488 BAPTA-1 AM (OGB 488, 0.79 mM). To improve membrane perforation of the dyes, a final concentration of 0.4% of Pluronic F127 (dissolved in 20% in DMSO) was added into AM dye solutions. In addition, Fluo-4 pentapotassium salt (1.08 mM) and Ca green dextran (1%) were also tested.

After dye loading, a DRG was moved to a custom-built recording chamber or a MatTek culture dish, held down with a nylon mesh, and superfused with the oxygenated extracellular solution.  $\text{Ca}^{2+}$  imaging experiments were performed under a Nikon confocal microscope with a 20 $\times$  objective or a custom-built two-photon laser-scanning microscope based on a Coherent Laser System with a water immersion 60 $\times$  objective. For the confocal microscope, the excitation wavelength was 488 nm. For the two-photon microscope, the laser system was operated at 800 nm.

The  $\text{Ca}^{2+}$  images were recorded before, during, and after an electrical sciatic nerve stimulation or a bath application of chemicals, e.g., high KCl (80 mM or 55 mM) or capsaicin (10  $\mu\text{M}$ ). For electrical stimulation, the cut end of the sciatic nerve was inserted into a suction electrode and connected to a Master-8 stimulator through an isolator. The stimuli were 1 ms square pulses,  $\leq 7$  mA, delivered at 20 Hz. Images were analyzed off-line with a NIH ImageJ software or Nikon NIS

Elements software. Data are presented as the relative change in fluorescence ( $\Delta F/F_0$ ), where  $F_0$  is basal fluorescence and  $\Delta F = F - F_0$ .

### Data analyses

Data were expressed as means  $\pm$  SE or as percentages. Student's *t* and chi square tests were used to access the significance of changes. The Mann–Whitney Rank Sum Test was used when the normality test failed for the Student's *t* test. Comparisons between multiple means were done with one-way analysis of variance followed by the Holm–Sidak *post hoc* test. A value of  $P < 0.05$  was considered significant.

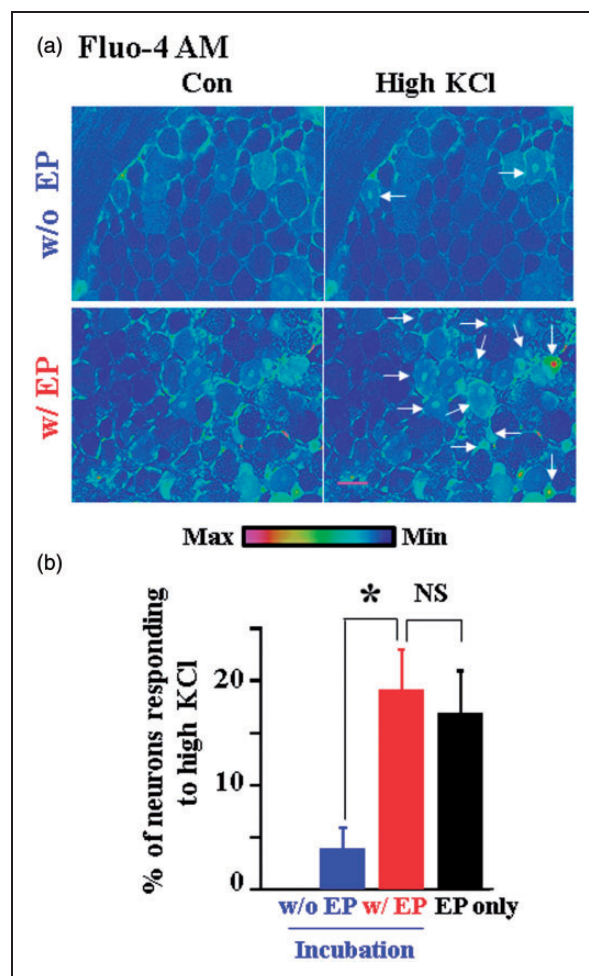
## Results

### Electroporation significantly improves the loading of Fluo-4 AM $Ca^{2+}$ indicator into DRG neurons

In our previous studies of neuron–glia interactions,<sup>5,6</sup> we loaded Fluo-4 AM into neurons and glial cells in whole DRGs through 1 h incubation period. The method allowed us to load Fluo-4 AM into glia. In contrast, only a limited number of neurons were loaded. To improve the  $Ca^{2+}$  dye loading in neurons, DRGs first underwent electroporation, followed by 1 h dye incubation. We found that electroporation greatly increased dye loading in neurons but had little effect on loading in glial cells (Figure 1). To further confirm that the electroporation-treated labeled neurons are functional, we studied the effect of electroporation on the number of labeled neurons in response to a high concentration (80 mM) of KCl treatment. KCl is known to reliably depolarize neurons causing  $Ca^{2+}$  influx into functional neurons.<sup>18</sup>

Electroporation followed by incubation was found to increase Fluo-4 AM labeled neurons responding to high KCl by 4.9 fold (Figure 1(b)). In addition, we found that Fluo-4 AM can be efficiently loaded into neurons through electroporation without postincubation (Figure 1(b)). These results suggest that electroporation significantly improves Fluo-4 AM loading into DRG neurons in intact DRGs. On the other hand, AM dye incubation can label glia well without electroporation.

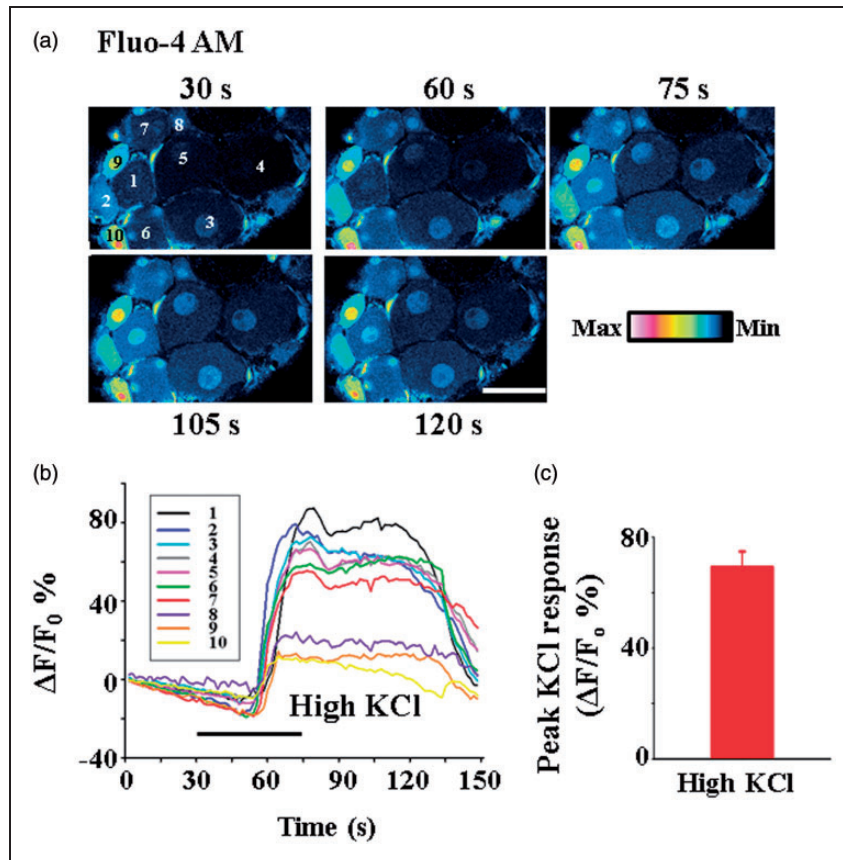
The improvement of dye loading also allowed us to study the time-dependent changes in intracellular  $Ca^{2+}$  in different neurons in intact DRGs. Following electroporation and incubation of Fluo-4 AM, we determined the dynamic changes in fluorescence in response to high KCl treatment (Figure 2). The fluorescence intensity peaked  $\sim 35$  s after high KCl application. The average peak fluorescence  $Ca^{2+}$  response ( $\Delta F/F_0$ ) was increased by  $\sim 69\%$  (Figure 2).



**Figure 1.** Electroporation increases Fluo-4 AM loading in intact DRG neurons. (a) Examples of pseudocolor intracellular  $Ca^{2+}$  images in cells responding to bath application of 80 mM KCl in intact DRGs with or without electroporation (EP) of Fluo-4 AM, followed by 1 h dye incubation. Arrows indicate the neurons responding to high KCl treatment. Scale bar: 50  $\mu$ m. Color-coded intensity calibration bar is shown below. (b) Electroporation increased the number of DRG neurons responding to high KCl stimulation ( $3.90 \pm 2.10\%$ , w/o EP,  $n = 3$  DRGs;  $19.10 \pm 3.86\%$ , w/EP;  $n = 4$ ;  $*P < 0.05$ ). In the absence of postincubation, electroporation of Fluo-4 AM yielded a similar percentage of neurons responding to KCl ( $16.90 \pm 0.04\%$  EP only,  $n = 4$  DRGs;  $P > 0.05$  compared with EP + incubation). Note that glia were labeled well with 1 h Fluo-4 AM incubation. Electroporation of Fluo-4 AM had minimal effect on glia labeling.

### Electroporation also improves loading other AM and salt forms of $Ca^{2+}$ dye in DRG neurons

To determine whether neuronal dye loading by electroporation is dye dependent, a number of other  $Ca^{2+}$  dyes were tested. We found that electroporation of OGB-488 AM was equally efficient in producing functional dye loaded DRG neurons (Figure 3(a)). In previous studies,



**Figure 2.** Dynamic changes in intracellular  $\text{Ca}^{2+}$  in response to high KCl in intact DRG neurons loaded with Fluo-4 AM. (a) Example of  $\text{Ca}^{2+}$  changes in the neurons in response to bath application of 80 mM KCl. The time in each frame indicates the time at which the image was taken. Scale bar: 40  $\mu\text{m}$ . (b) Time-dependent changes of intracellular  $\text{Ca}^{2+}$  in DRG neurons in response to 80 mM KCl application. The line numbers correspond to the cell numbers labeled in (a). (c) The average peak fluorescence  $\text{Ca}^{2+}$  response ( $\Delta F/F_0$ ) induced by 80 mM KCl in all the responding DRG neurons ( $n=76$ ) in this experiment is  $69.00 \pm 5.00\%$ .  $F_0$  is the basal fluorescence before KCl stimulation.

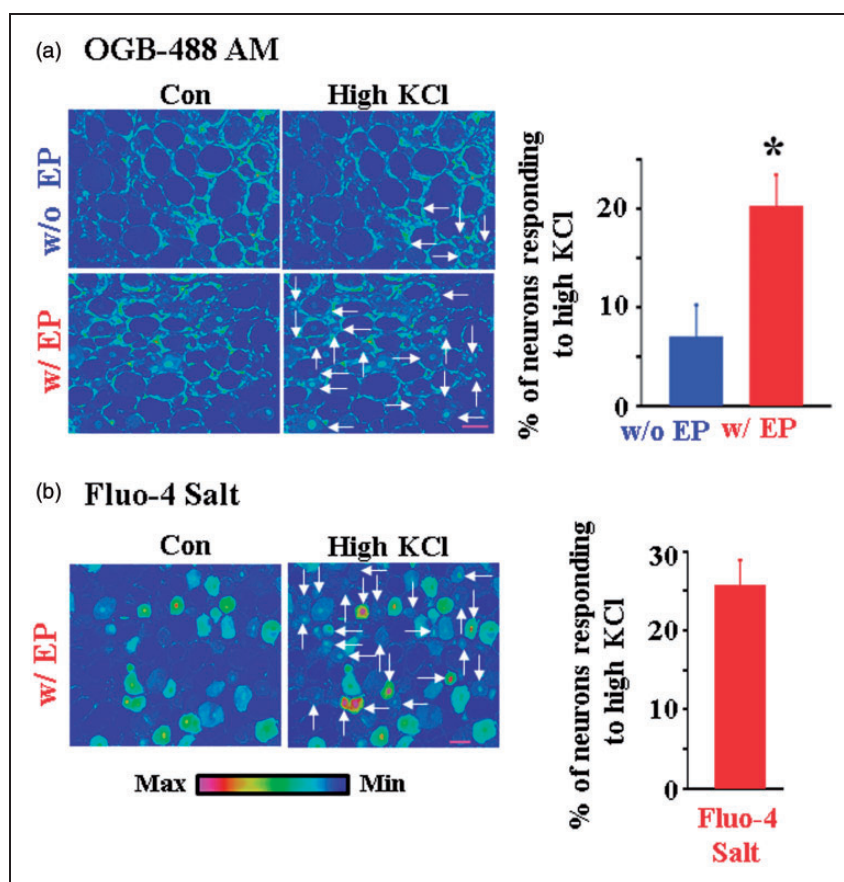
a salt form  $\text{Ca}^{2+}$  indicator, e.g., Fluo-4 pentapotassium, was loaded into a small number of neurons by direct intracellular injection.<sup>19</sup> About 26% of neurons responded to high KCl following electroporation (Figure 3(b)). In contrast to  $\text{Ca}^{2+}$  AM dyes, the loading of Fluo-4 salt into glia was rather limited.

We also determined changes in intracellular  $\text{Ca}^{2+}$  fluorescence in L4 or L5 DRG neurons in response to electrical stimulation of sciatic nerves. The DRG preparation has been electroporated and then incubated with OGB488 AM. Following electric stimulation, the  $\text{Ca}^{2+}$  fluorescence in neurons robustly increased and reached to its peak within 1–2 s. The fluorescence quickly dropped when the stimulation was stopped and then gradually returned to the baseline (Figure 4). We found that the average peak increase was  $35.00 \pm 5.00\%$  ( $\Delta F/F_0$ ;  $n=5$ ). These results suggest that both axons and somata of DRG neurons are functional following electroporation. Using Fluo-4 AM, we previously

showed that neurons and glia interact.<sup>5,6</sup> To determine whether neuron–glia interactions are maintained when DRGs undergo electroporation,  $\text{Ca}^{2+}$  changes in glia were also determined. Glial  $\text{Ca}^{2+}$  was increased with a delay (Figure 4), similar to our studies without electroporation.<sup>5</sup> Thus, neuron–glia interactions are indeed preserved.

We repeated the experiments using high KCl stimulation (Figure 5). As expected, the rates of  $\text{Ca}^{2+}$  fluorescence increase in neurons and glia following chemical, e.g., KCl, stimulation are slower than those following electrical stimulation. The delay in  $\text{Ca}^{2+}$  fluorescence increase between neurons and glia persisted. Following CFA, neuron–glia interactions were preserved, and the interactions occurred in more neuron–glia cell pairs. Six neuron–glia interactions were found in 76 KCl responsive neurons (7.9%) in normal rats, and 11 interactions were seen in 46 KCl responsive neurons (23.9%) in CFA rats.





**Figure 3.** Electroporation improves loadings of OGB-488 AM and Fluo-4 salt in intact DRG neurons. (a) Electroporation of OGB-488 AM significantly increased the number of DRG neurons in response to 80 mM KCl ( $7.01 \pm 3.19\%$  w/o EP,  $n = 4$  DRGs;  $20.30 \pm 3.13\%$ , w/EP;  $n = 10$ ;  $P < 0.05$ ). Note that many glia were labeled with the dye. Scale bar: 50  $\mu\text{m}$ . (b) Electroporation also promoted the loading of Fluo-4 salt in DRG neurons. High KCl evoked an increase in fluorescence intensity in many DRG neurons ( $25.80 \pm 3.13\%$ ;  $n = 4$  DRGs). In contrast, the number of glia labeled by Fluo-4 salt was limited. Scale bar: 50  $\mu\text{m}$ .

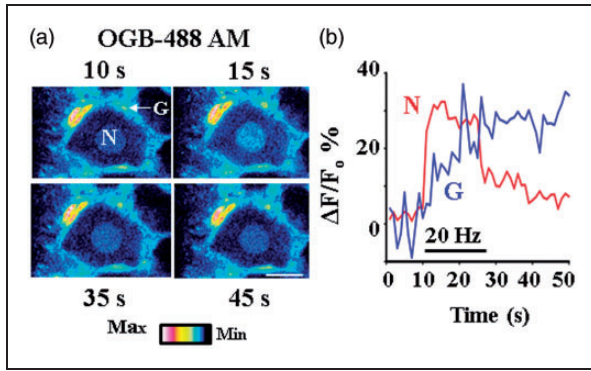
### Dextran $\text{Ca}^{2+}$ indicators respond to high KCl stimulation in very few rat DRG neurons

One exception to the success in functional preservation using electroporation was the use of dextran form  $\text{Ca}^{2+}$  indicator, i.e., Ca green dextran. Although we were able to load the dextran dye in intact DRGs, only a very low percentage of neurons ( $3.72 \pm 0.42\%$ ;  $n = 5$ ) responded to high KCl treatment (Figure 6(a)). We also used retrograde transport instead of electroporation to load Ca green dextran in DRGs. Neurons with retrograde loaded dye did not respond to high KCl treatment (Figure 6(b)). Thus, the failed KCl experiments are not dye loading method dependent. Others have reported that in adult rats none of the DRG neurons that underwent retrograde loading of Ca green dextran responded to sciatic nerve electric stimuli or bath application of high KCl.<sup>20</sup> Since

peripheral or CNS neurons loaded with dextran  $\text{Ca}^{2+}$  dyes do respond to manipulations such as KCl or electrical nerve stimulation,<sup>21–23</sup> the observations suggest that dextran  $\text{Ca}^{2+}$  dyes are not suitable for studying  $\text{Ca}^{2+}$  functions in DRG neurons. Reasons underlying the failure are unclear.

### Electroporation facilitates studies of pain signaling processing in intact DRG neurons

To determine whether the electroporation technique facilitates the study of pain signaling processing in intact DRG neurons, we studied the effect of capsaicin in DRGs treated with electroporation of Fluo-4 salt. Capsaicin was found to increase  $\text{Ca}^{2+}$  concentrations in many small and medium DRG neurons (Figure 7(a)), consistent with the studies obtained from the distribution of capsaicin receptors in DRGs.<sup>24–27</sup>



**Figure 4.** Neuron–satellite glial cell interactions triggered by electrical nerve stimulation. Sciatic nerve stimulation at 20 Hz increased intracellular  $\text{Ca}^{2+}$  in DRG neurons and glial cells. The DRG with attached sciatic nerves was electroporated and incubated with OGB 488-AM. (a) Pseudocolor images of fluorescence changes in a neuron (N) and a glial cell (G). The number at the top or bottom in each frame indicates the time at which the image was taken. Scale bar: 20  $\mu\text{m}$ . (b) Time courses of relative fluorescence changes, i.e.,  $\Delta F/F_0$ , in the neuron and glial cell.  $F_0$  is the basal fluorescence in either the neuron or the glial cell before nerve stimulation. The horizontal line indicates the period of nerve stimulation. Nerve stimulation evoked intracellular  $\text{Ca}^{2+}$  increase in the neuron first and then in the glial cell with a delay of  $\sim 2.0$  s.

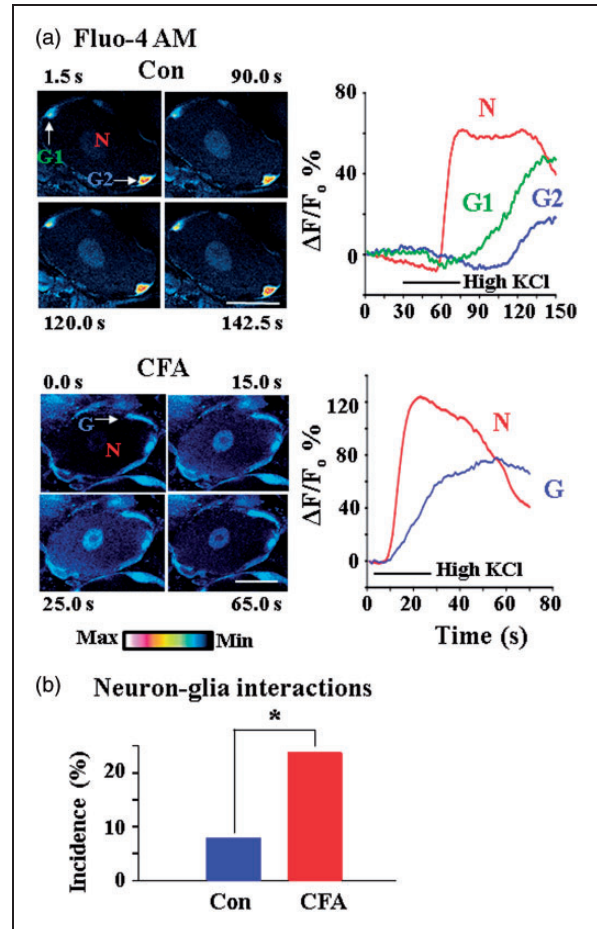
Thus, the increase in  $\text{Ca}^{2+}$  dye loading with electroporation is likely to help us characterize functions of capsaicin receptor activation in intact DRG neurons.

We also studied the morphine effects on DRG neurons underwent electroporation with Fluo-4 AM (Figure 7(b)). The effects of morphine on KCl-induced  $\text{Ca}^{2+}$  increases in cells treated with and without morphine were studied. Morphine was found to block the KCl-induced  $\text{Ca}^{2+}$  fluorescence in DRG neurons by  $\sim 80\%$ , a result consistent with the observation that morphine blocks voltage-dependent  $\text{Ca}^{2+}$  channels in DRG neurons.<sup>28</sup>

## Discussion

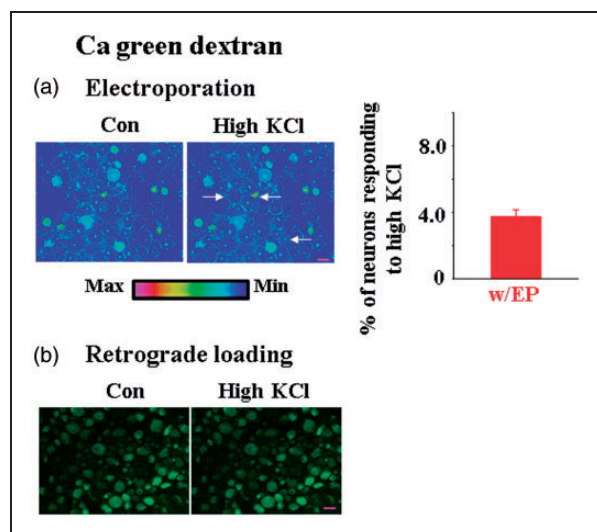
In this study, we found that electroporation of intact DRGs greatly facilitates the loading of  $\text{Ca}^{2+}$  dyes simultaneously into individual neurons without affecting their physiological functions. By combining electroporation and incubation of AM  $\text{Ca}^{2+}$  dye, we were able to study neuron–glia interactions efficiently in intact DRGs. The major advantages of this approach are its simplicity and high efficiency.

Bulk AM  $\text{Ca}^{2+}$  dye loading of CNS neurons has been successful in *in vitro* and *in vivo* preparations.<sup>29–31</sup> It is unclear the reason for poor loading of AM  $\text{Ca}^{2+}$  dye in intact DRG neurons in the absence of electroporation. One of the possibilities is that each soma of DRG neuron



**Figure 5.** Inflammation increases the incidence of neuron–glia interactions. Inflammation was induced by injecting CFA into the left hindpaw of a rat. DRGs from control and CFA rats were taken out and electroporated and incubated with Fluo-4 AM. (a) Example pseudocolor images of intracellular  $\text{Ca}^{2+}$  changes in DRG neurons from a control (upper) and a CFA (lower) rat in response to bath application of 80 mM KCl. High KCl increased the intracellular  $\text{Ca}^{2+}$  first in the neuron (N) and then in glial cells (G1 and G2) with a delay. The number at the top or bottom in each frame indicates the time the image was taken. Time courses of relative fluorescence changes, i.e.,  $\Delta F/F_0$ , in the neuron and in the glial cells are shown on the right. The horizontal lines indicate the period of high KCl application. For the images of the CFA rat, high KCl induced similar responses with those in the control rat both in the neuron and in the glial cell. Scale bars: 30  $\mu\text{m}$ . (b) Neuron–glia interactions were significantly increased in DRGs of CFA rats. In response to high KCl stimulation, 7.9% KCl responsive neurons interacted with glia in control and 23.9% in CFA rats interacted.

is tightly surrounded by a layer of SGCs, which form a functional unit enclosed by a connective tissue sheath.<sup>32</sup> This results in a physical barrier for  $\text{Ca}^{2+}$  dye to gain access to DRG neurons.

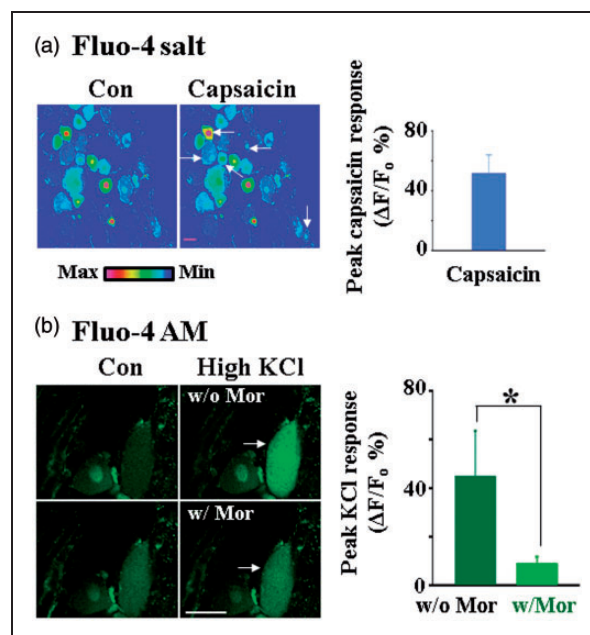


**Figure 6.** Few DRG neurons loaded with the dextran form of  $\text{Ca}^{2+}$  dyes are functional. (a) Bath application of 80 mM KCl evoked intracellular  $\text{Ca}^{2+}$  changes in a limited number of neurons in DRGs that underwent electroporation of Ca green dextran ( $3.72 \pm 0.42\%$ ,  $n = 5$  DRGs). Arrows indicate the responding neurons. Scale bar: 50  $\mu\text{m}$ . (b) In another rat, true color cell images indicated that none of the DRG neurons retrogradely loaded Ca green dextran responded to high KCl ( $n = 3$  DRGs). Scale bar: 50  $\mu\text{m}$ .

The number of neuron–glia interacting pairs induced by high KCl is increased in DRGs in CFA-induced inflamed rats (Figure 5). We have previously shown that CFA-induced inflammation enhances the expression of P2X7 receptors in rats, and the neuron–glia interactions depend on the P2X7 receptors in SGCs in DRGs.<sup>5,6</sup> The roles of P2X7R in neuron–glia interactions in CFA rats can now be further determined with the improved  $\text{Ca}^{2+}$  dye loading in intact DRGs.

The results that capsaicin causes an increase in  $\text{Ca}^{2+}$  signaling in small and medium cells and the  $\mu$ -opioid receptor agonist, morphine, significantly inhibits high KCl-induced  $\text{Ca}^{2+}$  increase in DRG neurons are consistent with published findings.<sup>33–36</sup> These observations suggest that the electroporation  $\text{Ca}^{2+}$  dye loading method should be useful for studying drug effects in intact DRGs in pain research.

Recently, genetic  $\text{Ca}^{2+}$  sensors have become available for studying  $\text{Ca}^{2+}$  changes in virus-transfected neurons and/or in mutant mice.<sup>37–39</sup> Using these sensors, it is possible to probe the  $\text{Ca}^{2+}$  activities in specific subpopulations of sensory neurons.<sup>40</sup> However, these approaches usually take weeks or months to produce results. Compared with genetic approaches,  $\text{Ca}^{2+}$  dye loading through electroporation and incubation cannot target specific populations of neurons. On the other hand, in combination with specific pharmacological tools,



**Figure 7.** Applications of intact DRG  $\text{Ca}^{2+}$  imaging in pain research. (a) Bath application of 10  $\mu\text{M}$  capsaicin evoked intracellular  $\text{Ca}^{2+}$  fluorescence changes in many small and medium DRG neurons with an average peak response of  $51.70 \pm 12.2\%$ , ( $\Delta F/F_0$ ,  $n = 14$  neurons). DRGs were electroporated with Fluo-4 salt. Scale bar: 25  $\mu\text{m}$ . Arrows indicate the neurons with responses. (b) Bath application of 35  $\mu\text{M}$  morphine inhibited 55 mM KCl-induced  $\text{Ca}^{2+}$  increase in DRG neurons. DRGs were electroporated and incubated with Fluo-4 AM. Left: Examples of true color images showing the inhibitory effect of morphine on the intracellular  $\text{Ca}^{2+}$  level in a neuron (arrow). Right: Morphine inhibited high KCl-induced  $\text{Ca}^{2+}$  increase in DRG neurons ( $\Delta F/F_0$ :  $45.00 \pm 18.50\%$  w/o morphine,  $9.19 \pm 2.52\%$  w/morphine,  $n = 10$ ). Scale bar: 25  $\mu\text{m}$ .

electroporation and incubation of  $\text{Ca}^{2+}$  dyes can efficiently provide general ideas about  $\text{Ca}^{2+}$  signaling in subpopulations of DRG neurons. We can then use the observations as a prelude to understanding  $\text{Ca}^{2+}$  signaling in a specific subpopulation of DRG neurons using genetic  $\text{Ca}^{2+}$  sensors.

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### Author Contributions

YC and LMH designed research. YC performed experiments. YC and LMH wrote the paper.

### Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.



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