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Prolonged Pain Research in Mice: Trends in Reference to the 3Rs

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ABSTRACT

This literature review documents trends in the use of mice in prolonged pain research, defined herein as research that subjects mice to a source of pain for at least 14 days. The total amount of prolonged pain research on mice has increased dramatically in the past decade for the 3 pain categories examined: neuropathic, inflammatory, and chronic pain. There has also been a significant rise in the number of prolonged mouse pain studies as a proportion of all mouse studies and of all mouse pain studies. The use of transgenic mice has also risen significantly in prolonged pain research, though not as a proportion of all mice used in prolonged pain research. There has not been significant overall change in the number of mice being used per study for any of the 3 pain categories or for any of 3 common pain inducement models: chronic constriction injury, partial sciatic nerve ligation, and complete Freund's adjuvant. Finally, although most authors referred to approval of experiments by an institutional nonhuman animal use committee, there were no references to the "3Rs" in a random selection of 55 papers examined. Given the proportionally high volume of mice used in invasive research and the gravity of studies that inflict lasting pain, these trends raise serious questions about whether the 3Rs principles of Replacement, Reduction, and Refinement are being appropriately implemented by researchers and institutions.

INTRODUCTION

The year 2009 marked the 50th anniversary since publication of the landmark text *The Principles of Humane Experimental Technique* by William Russell and Rex Burch (1959). Russell and Burch's book spawned "the 3Rs," a widely accepted ethical framework for the use of nonhuman animals in research, testing, and education. The 3Rs framework seeks Replacement of animals with nonanimal methods, Reduction in the number of animals used, and Refinement to minimize animal pain and suffering. The avoidance of pain and its treatment with analgesics are paramount priorities in animal research (National Research Council [NRC], 2007).

By some measures, the 3Rs have yielded progress, especially in the arena of animal testing. For example, there has been a movement toward in vitro and computational methods in toxicology testing following a 2007 landmark report by the U.S. National Research Council (NRC, 2007). This report represents a significant paradigm shift; there have also been important changes to specific testing protocols. Figures are not readily available, but The Lethal Dose 50% (LD50) test has been altered so that it causes suffering to fewer animals; the "up-and-down" method reduces by more than 70% the number of animals required (Lichtman, 1998) and is estimated to have spared at least 10,000 mice from the LD50 test over the past decade (M. Stephens, Johns Hopkins University Center for Alternatives to Animal Testing, personal communication, April 15, 2011). In the area of research, replacement of the painful ascites method with nonanimal methods for the production of monoclonal antibodies (McArdle, Reddington, Reddington, & Heidel, 1996) has spared more than 2 million mice yearly worldwide. Strides have also been made in the areas of vaccine potency and biologics testing, among others.

However, other reported trends suggest notable failures in 3Rs implementation (Taylor, Gordon, Langley, & Higgins, 2008). The use of animals in pain experiments is an area of special welfare concern because animals are deliberately subjected to pain-inducing procedures, and pain relief is typically withheld as part of the study design. The number of animals used in pain experiments has increased significantly over the past several decades. Between 1963 and 2007, the number of published papers describing pain experiments in animals increased by more than 20-fold (Mogil, 2009). Although the number of studies using dogs, cats, rabbits, and other animals in pain experiments has remained relatively stable with some fluctuations, there has been a dramatic increase in the number of published studies of rats and mice in pain experiments (Mogil, 2009).

Mice (*Mus musculus*) in the laboratory comprise a large proportion of the estimated 100 million vertebrate animals currently used worldwide for animal experimentation each year (Taylor et al., 2008). By our own estimates, the number of mouse pain

studies averaged fewer than 5 per year between 1963 and 1976, increased to at least 30 studies per year by 1991, and has expanded to over 200 studies per year since 2006. In 2010, the number of pain studies using mice decreased slightly for the first time since 2003.

The aim of this study was to quantify trends in the use of laboratory mice, including transgenic mice, in a subset of pain research that is of particular concern for animal welfare: studies in which animals are subjected to prolonged pain. We addressed six questions:

1. Has the total amount of research using mice in prolonged pain research changed through time?
2. Has the proportion of studies using mice in prolonged pain research changed relative to the total number of mice used in research or mice used in pain research?
3. Has the use of transgenic mice in prolonged pain research changed through time?
4. Has the number of mice per study being used in prolonged pain research changed through time?
5. Has the number of mice per study for a given pain model changed through time?
6. To what degree are researchers reporting efforts to implement 3Rs in their studies?

Questions 1 through 5 address the possibility of efforts at Reduction and/or Replacement of mice in prolonged pain research. Question 6 addresses possible efforts to implement all three Rs, including Refinement, in the use of mice in this research. Any declines in absolute or proportional numbers of mice being used can be seen as success in the areas of Reduction and Replacement. To date, a detailed analysis of such trends is lacking, and this analysis can provide a useful assessment of progress in implementing the 3Rs.

MATERIALS AND METHODS

Literature Searches

We conducted literature searches using PubMed, a free online version of MEDLINE that also includes up-to-date citations not yet indexed for MEDLINE. Date of publication was determined by date of printed journal in which the article appeared (i.e., not e-publication date for which electronic versions of papers may appear in the prior calendar year). We conducted searches using the string "mouse AND pain

AND [pain category]." The three pain categories we used were "neuropathic," "inflammatory," and "chronic."

Inclusion and Exclusion Criteria

Studies were selected for analysis based on the following two inclusion criteria: (a) studies involving mice (transgenic and/or wild-type) and (b) studies that sought to subject mice to prolonged pain (we used this term only to define the duration of pain and to avoid confusion with one of our pain categories, "chronic pain"). Prolonged pain studies were those in which mice were subjected to the source of pain for 14 or more days. Although there is no standard definition of what qualifies as prolonged pain in mice, we consulted three independent experts in the field of rodent pain research before conservatively selecting the 14-day criterion.

Studies were included in the analysis if any of the mice met the 14-day criterion. We excluded from our analysis studies not in English, studies of acute pain or stress (e.g., acute thermal or mechanical nociception), in vitro studies, and drug (including alcohol and tobacco) withdrawal studies. The most common reason for excluding a study from the analysis was that the source of pain lasted less than 14 days. Other types of studies omitted from our literature search included review papers, studies of rats, and in vitro studies.

Pain Research Categories

For the remainder of the analysis, we focused on three categories of pain research: neuropathic, inflammatory, and chronic pain. We used the search terms "mouse AND [category of] pain" in conjunction with each of these three categories, respectively. We found some overlap across these pain categories, such as when researchers induced both neuropathic and inflammatory pain in mice. In cases where a given study qualified for more than one pain category, we assigned it to the predominant one (based on numbers of mice used) to avoid counting a study more than once.

Methods of Pain Inducement

We used the methods sections of published papers to identify the primary method by which prolonged pain was induced in all qualifying studies.

Total Amount of Prolonged Mouse Pain Research (Question 1)

To address the total volume of prolonged pain studies being done on mice, we simply plotted the number of qualifying (prolonged mouse pain) studies by year.

Proportion of Studies Subjecting Mice to Prolonged Pain (Question 2)

An overall increase in the number of studies in which mice are being subjected to prolonged pain could reflect an increase in the number of researchers doing mouse

pain research. To address this possibility we examined the number of mice being used in prolonged pain studies as a proportion of all mouse studies and as a proportion of all mouse pain studies. We performed literature searches using the PubMed database and the search term “mouse” and compared the number of hits with the number of qualifying studies we identified for each year across all three pain categories. We also compared qualifying studies with a Boolean search for “mouse AND pain.” Because there were so few qualifying studies prior to 2000, we limited this analysis to the years 2000 to 2010.

Use of Transgenic Mice (Question 3)

We identified prolonged mouse pain studies in which one or more mouse strains used were transgenic. We assessed the yearly frequency of the number of these studies in comparison to the number of prolonged mouse pain studies using only nontransgenic mouse strains. We also recorded the number of prolonged pain studies using transgenic mice as a proportion of all prolonged mouse pain studies for a given year to see if any trends in relative use of transgenic mice emerged.

In a few studies, transgenic mice were reported to have suppressed pain responses; in others, pain responses were amplified. In our analysis we did not distinguish these factors on the grounds that suppressed pain response cannot be assumed to be an indication of less pain and vice versa.

Number of Mice Used per Study (Question 4)

To evaluate possible trends in the number of mice used per study, we randomly selected three qualifying studies per pain category (neuropathic, inflammatory, and chronic) for a given publication year and attempted to ascertain the number of mice used in those studies. In years for which fewer than three qualifying studies were published, we included all studies published that year in the analysis. Each paper was carefully reviewed to determine, as best as possible, the number of mice used. Methods sections, figures, and tables were the most likely places to find information on sample sizes. Authors often provided a range in numbers of mice used in various experiments; in these cases we estimated the minimum and maximum number of mice used and used the mean of these two estimates. Within a qualifying study, only those mice subjected to painful stimuli for 14 days or more were included in this analysis. Control groups were included if they were subjected to the pain inducement method (e.g., controls were a different strain of mouse) and excluded if they were not (e.g., sham-operated animals). Two authors (J. B., L. B.) independently conducted counts of a subsample of papers to establish concordance of the counting method.

Use of Mice in Specific Pain Models (Question 5)

We evaluated the possibility that researchers may be using fewer mice in prolonged pain studies for specific pain models. For each year we compared the average number of mice used in a random selection of up to five qualifying studies with the average number of mice used in up to five nonqualifying studies that used the same model. Nonqualifying studies were those flagged in original literature searches that did not to meet our inclusion criteria. We selected the three most commonly used pain models for this analysis: chronic constriction injury (CCI), partial sciatic nerve ligation (PSNL), and complete Freund's adjuvant (CFA). Sample sizes for some years were small, and because few applicable studies occurred before 2000, we used the average of all pre-2000 studies for a given category. In years for which there were three or more studies in a particular category, we randomly selected three studies.

We also compared the overall means of all individual mouse counts (all years combined) between qualifying and nonqualifying studies within each of the three pain models.

Reporting 3Rs Efforts (Question 6)

To evaluate the degree to which researchers are reporting efforts to implement 3Rs in their studies, we performed searches for the following keywords in selected papers: "3Rs," "Reduc[e][tion]," "Replace," "Refine," "Humane," "Minimize," "Guide[line]," and "Ethic." We also performed searches for language pertaining to committee approval. We randomly selected five papers per year for this analysis and pooled data for qualifying studies published before 2000 ($n = 9$).

We recorded whether each given term appeared at least once in the published paper. We took care to include terms only when they were used in the context of relieving animal pain and/or suffering. Certain terms (especially "reduce") often occurred out of this context.

Other Trends

We compiled a list of the different methods used to induce prolonged pain in mice. We also noted the countries in which the research was carried out.

Statistics

Nonnormal distributions of data, and in some cases small sample sizes, favored the use of nonparametric statistics in this study. To evaluate whether or not the proportion of studies using mice in prolonged pain research has changed through time (Question 2), we used the Jonckheere-Terpstra test for trend (Jonckheere, 1954), a nonparametric test for the null hypothesis that the distribution of a data

set does not differ across time (in our study, years). We also used the Jonckheere test to assess the possibility of yearly trends in the use of transgenic mice (Question 3).

We used the Spearman's rho correlation test to assess the possibility of yearly trends in the number of mice being used per study for each pain category (Question 4) and for numbers of mice being used per study within our three selected pain inducement models (Question 5). We used the Wilcoxon test to compare overall means for the three pain inducement methods.

To evaluate the reporting of efforts to implement 3Rs in studies of prolonged mouse pain (Question 6), we used the Jonckheere-Terpstra test for trend, which accommodates small sample sizes.

Because so few qualifying studies were published before 2000, and because we were applying nonparametric statistics to the data, studies published before 2000 were pooled for the trends analysis of the number of mice per study (Question 5).

RESULTS

Methods of Inducing Pain

A wide variety of methods are used to induce prolonged pain in mice and other animal subjects of pain research (Table 1). We identified 26 different methods used in the studies we examined. The most commonly used of these methods fell under the neuropathic pain category and involved variations on the theme of eliminating or compromising a given nerve by transection, ligation, or partial ligation. In particular, CCI and PSNL have remained popular since their initial development in 1988 (Bennett & Xie, 1988) and 1990 (Seltzer, Dubner, & Shir, 1990), respectively. We found that the administration of CFA is another commonly used method of inducing prolonged inflammatory pain in mice (Chillingworth & Donaldson, 2003).

Total Amount of Prolonged Mouse Pain Research (Question 1)

We identified a total of 320 studies published between 1979 and 2010 that met our inclusion criteria for prolonged pain. The volume of studies focused on neuropathic models of pain (n = 201) was substantially higher than models of chronic pain (n = 70) or inflammatory pain (n = 49; Figure 1). The three most recent years of our analysis (2008–2010) yielded more studies (202, 63%) than all previous years combined (118, 37%). Most of this recent increase is attributable to neuropathic pain studies; the volume of prolonged pain research using mice in inflammatory and chronic pain models has remained fairly stable since 2006.

Table 1. Common methods used to induce prolonged pain in mice and rats

Method	Predominant Pain Category	Number of Studies
Chronic constriction injury	Neuropathic	50
Partial sciatic nerve ligation	Neuropathic	39
Complete Freund's adjuvant	Chronic	30
Sciatic nerve ligation or injury	Neuropathic	29
Spared nerve injury	Neuropathic	28
Spinal nerve ligation or injury	Neuropathic	24
Other nerve injuries (saphenous, infraorbital, etc.)	Chronic	21
Injections of painful compounds	Inflammatory	19
Diabetes (usually via streptozotocin)	Neuropathic	17
Bone cancer (tumor cell implantation)	Chronic	15
Peripheral nerve injury	Neuropathic	11
Arthritis	Inflammatory	8
Colitis	Inflammatory	4
Colorectal distension	Inflammatory	4
Endometriosis	Inflammatory	3
Schistosomiasis	Chronic	2
Surgical pain	Chronic	2
Viral infection (e.g., herpes)	Chronic	2
Dental pain (drilling)	Inflammatory	1
Induced fibromyalgia	Neuropathic	1
Tail vertebra compression	Inflammatory	1
Fish venom	Inflammatory	1
Chronic pancreatitis	Chronic	1
Amyotrophic lateral sclerosis	Inflammatory	1
Temporal-mandibular joint	Inflammatory	1
Human sickle hemoglobin	Neuropathic	1
Total		316

Proportion of Studies Subjecting Mice to Prolonged Pain (Question 2)

We found strong evidence of a trend in the number of prolonged mouse pain studies as a proportion of all mouse studies ($p < .0001$ using Jonckheere-Terpstra test) and as a proportion of all mouse pain studies ($p < .0001$) for years 2000 to 2010 (Figure 2).

Use of Transgenic Mice (Question 3)

The overall use of transgenic mice in prolonged pain research has increased over time ($p < .0001$ using Jonckheere-Terpstra for years 2000–2010; Figure 3). However, when taken as a proportion of all prolonged mouse pain studies, we did not find a significant trend in the use of transgenic mice in this body of research (Jonckheere-Terpstra $p = .45$ for years 2000–2010; Figure 4).

Figure 1. Number of prolonged pain studies using mice in three categories of pain research (neuropathic pain, inflammatory pain, and chronic pain) based on PubMed MeSH search conducted on April 26, 2011.

Number of Mice Used per Study (Question 4)

Ascertaining the number of animals used in a study and the number of mice subjected to painful stimuli versus control animals was difficult for many of the articles we reviewed. In about 20% of all papers examined, sample sizes were clearly stated. However, in most cases, mouse counts required careful examination of the paper. Results sections, and especially figure captions, were the most likely places to find sample sizes, but even then the data were often too vague to ascertain exact numbers.

Typically, between 40 and 80 mice were subjected to prolonged pain in a given study (Figure 5), but the range was from fewer than 10 to several hundred mice. We did not find statistically significant trends across years in the number of mice being subjected to prolonged pain in any of the three pain categories. Spearman's rho p values were .70 for neuropathic pain studies, .07 for inflammatory pain studies, and .10 for chronic pain studies.

Figure 2. Number of prolonged mouse pain studies as a proportion of (a) all mouse studies and (b) all mouse pain studies.

(a)

(b)

Use of Mice in Specific Pain Models (Question 5)

We did not find significant trends in the number of mice used per study for the three most commonly used pain models we examined (Figure 6). Jonckheere-Terpstra trend analyses produced the following results: CCI qualifying studies, $p=.18$, $n=30$, nonqualifying studies, $p=.21$, $n=12$; PSNL qualifying studies, $p=.69$, $n=23$, nonqualifying studies, $p=.29$, $n=26$; and CFA qualifying studies, $p=.35$, $n=13$, nonqualifying studies, $p=.48$, $n=9$.

Figure 3. Number of prolonged mouse pain studies using transgenic mice published since 2000.

Figure 4. Number of prolonged mouse pain studies using transgenic mice as a proportion of all prolonged pain studies.

Figure 5. Number of mice per study subjected to prolonged pain by pain category. $n=3$ for each bar from 2004 onward; $n \leq 3$ for each bar preceding 2004.

Figure 6. Mean numbers of mice per study for qualifying (prolonged pain) and nonqualifying studies by pain model. Each bar represents the yearly mean number of mice used per study. (a) CCI = chronic constriction injury model; (b) PSNL = partial sciatic nerve ligation (note different vertical scale); (c) CFA = complete Freund's adjuvant. q = qualifying studies; n = nonqualifying studies.

(a)

(b)

(c)

The distributions of the number of mice between qualifying and nonqualifying studies did not differ for any of the three pain models. Wilcoxon tests yielded $p = .41$ for CCI studies, $p = .31$ for PSNL studies, and $p = .22$ for CFA studies (Figure 7).

Reporting 3Rs Efforts (Question 6)

None of the 55 studies used in this analysis reported any of the following keywords directly associated with the 3Rs framework: 3Rs, Reduce, Replace, or Refine. The terms humane, minimize, and ethic were mentioned in 2 (4%), 4 (7%), and 9 (16%) studies, respectively. Many authors (24 studies, 44%) referred either to a guideline or guide, and a majority (39, 71%) referred to approval of the protocols by an institutional animal use committee (Figure 8).

Figure 9. Comparison of mean (+SD) numbers of mice used per study for qualifying (prolonged pain) and nonqualifying studies by pain model. Each bar represents the mean of all studies in each category. CCI = chronic constriction injury model; PSNL = partial sciatic nerve ligation; CFA = complete Freund's adjuvant; Q = qualifying studies; NQ = nonqualifying studies. Sample sizes are in parentheses.

There was not a trend through time for any of the aforementioned terms, with the exception of guideline, which showed an increasing proportion of studies through time (Jonckheere-Terpstra trend test $p = .0017$).

Figure 8. Proportion of yearly sample of published studies in which specific keywords were mentioned in the context of the 3Rs (Reduce, Refine, or Replace). Each bar represents a proportion of five studies with the exception of 2000 (three studies) and 2002 (two studies). No studies mentioned the 3Rs, so they are not shown here.

Geographic Patterns of Use

The studies qualifying for inclusion in our general analysis were conducted in a total of 27 different countries (Table 2). The United States led the list with 106 studies. Japan (62 studies), Germany (39), the United Kingdom (34), Canada (20), and Italy (14) rounded out the remaining top 5 countries. Europe was represented by 14 countries (including Turkey); Asia by 6 (including the Ukraine); and there were 2 each from North America and the Middle East (Israel and Iran) and 1 each from Africa (Cameroon), Latin America (Brazil), and Australia.

DISCUSSION

Research that inflicts pain, particularly research that causes prolonged unrelieved pain, is of special concern from an ethical perspective. Our analysis documents both an absolute and a proportional rise in the total volume of prolonged mouse pain studies during the past 15 years and in particular a dramatic rise in research that subjects mice to prolonged neuropathic pain during the last 3 years of the analysis (2008–2010). These trends illustrate a failure in one of the 3Rs: Replacement.

Notwithstanding these overall increases, the most recent years' data suggest a possible plateau in the volume of total mouse prolonged-pain research. It has been suggested that pain researchers may be reverting to the use of rats, which were the favored subjects of animal pain research before mice came to the fore (Colleoni & Sacerdote, 2009; Mogil 2009). If so, this would not be a success from the 3Rs perspective.

Table 2. Number of Qualifying Studies by Country

Country	Neuropathic Pain	Inflammatory Pain	Chronic Pain	Total
United States	54	19	33	106
Japan	50	7	5	62
Germany	30	2	7	39
United Kingdom	28	2	4	34
Canada	8	5	7	20
Italy	14	1	3	18
Spain	9	2	3	14
France	10	1	2	13
Brazil	5	5	1	11
South Korea	5	3	2	10
Sweden	5	1	1	7
Belgium	4	1	1	6
China	6	0	0	6
Poland	4	1	1	6
Switzerland	6	0	0	6
Israel	2	0	2	4
Denmark	3	0	0	3
Hungary	1	1	1	3
India	1	0	2	3
Taiwan	1	1	1	3
Australia	1	0	1	2
Austria	1	0	1	2
Iran	0	0	2	2
Cameroon	1	0	0	1
Netherlands	1	0	0	1
Turkey	1	0	0	1
Ukraine	0	1	0	1
Total	251	53	80	384

Note: Some studies were represented by more than one country.

It is interesting to note that although the use of transgenic mouse strains in prolonged mouse pain research has risen steadily over the past decade, the proportion of studies using transgenic mice has not grown. Thus, the proportional rise in prolonged mouse pain research is not solely attributable to an influx of transgenic models. Nevertheless, there has been a proliferation of transgenic mouse strains, and as of 2009 there were 274 different knockout mice reported to have pain phenotypes (Colleoni & Sacerdote, 2009; Mogil, 2009). We caution against interpreting the use of transgenic mice as constituting a Refinement; some transgenic strains have shown behavioral evidence for enhanced (e.g., Helyes et

al., 2009; Racz et al., 2008), and some reduced (e.g., Chessell et al., 2005; Hughes, Hatcher, & Chessell, 2007), sensitivity to pain.

A drop in the number of mice used per study might mitigate the rising numbers and proportion of studies using mice and would represent a measure of success in meeting one of the 3Rs: Reduction. We found no such trend across any of the three pain categories we examined (neuropathic, inflammatory, and chronic). Thus, there does not appear to be any direct effort on the part of prolonged mouse pain researchers to implement reductions in the numbers of animals they are using.

By evaluating the number of mice used per study for three specific models of pain, we addressed the possibility that reduction efforts may be occurring in particular branches of pain research. Once again, we did not find evidence that the number of mice per study was declining through time for any of the three pain models examined. We also did not find any significant difference in the number of mice being used in studies that qualified as prolonged pain compared with studies subjecting mice to shorter periods of pain for any of the three specific pain models (e.g., chronic constriction injury). It is possible that pain researchers in other fields (e.g., cancer pain) are making measurable efforts to subject fewer mice to prolonged pain, but we did not find evidence of this for the pain categories we examined.

That the 3Rs or any of the 3Rs components—Replace, Reduce, or Refine—were not mentioned in any of 55 studies suggests that prolonged mouse pain researchers may be unaware of or indifferent to the 3Rs framework and that this aspect is not considered relevant in the peer review process of manuscripts for scientific journals. A small percentage of authors did mention efforts to minimize the number of animals and/or pain caused to animals in their studies. Our findings reflect a recent analysis of 250 research papers reporting experiments on primates and mice which found that reporting using the 3Rs is very low and not increasing (Taylor, 2011). Most often, authors referred to efforts to abide by a set of guidelines, such as those of the International Association for the Study of Pain (IASP, 2012), or approval by the researchers' institutional animal use committees.

Our finding that only the term "guideline" showed a significant increase in usage through time might suggest that adherence to guidelines by themselves is adequate to ensure 3Rs progress. However, the growing proportion of the number of studies subjecting mice to prolonged pain and the lack of any change in the number of mice being subjected to prolonged pain reported elsewhere in this paper suggests that adherence to guidelines and/or animal use committee requirements is not translating into significant progress from a reduction or replacement perspective.

The geographic distribution of prolonged pain research using mice reflects global patterns of overall animal use in research, so we did not find a disproportionate

distribution of prolonged mouse pain research per se. The United States is the world's largest user of animals in research, and the same is true for studies of prolonged pain in mice. The next five highest users—Japan, Germany, the United Kingdom, Canada, and Italy—all rank among the world's primary overall users of animals in experimentation.

There are limitations of our study. The dearth of qualifying studies published before 2004, and especially before 2000, hindered our ability to determine early trends in the number of mice being used per study because often the number of studies for a given pain category year was one or zero. The difficulty of estimating how many mice were used in a given study was another limitation. In some studies it is virtually impossible to determine the exact numbers of mice used, as noted by other authors (Hampson, Southee, Howell, & Balls, 1990).

CONCLUSION

Studies that induce prolonged pain are a high priority for the application of the 3Rs principles. However, our findings suggest that researchers conducting prolonged pain research on mice are paying little, if any, heed to 3Rs principles in the planning and execution of their research. The trends we present here constitute a measurable failure in two of the 3Rs established by Russell and Burch in their 1959 book: a failure to Replace animal-based methods with nonanimal methods and a failure to Reduce the number of animals being used.

Alternative approaches exist for replacing and reducing animal use and for refining methods to minimize pain and suffering, and this is a very active area of research (Goldberg, 2007). In the area of pain research, ethical clinical studies with human patients have the advantage that humans can reliably report their experience of pain and pain relief (Brown, Seymour, Boyle, El-Deredy, & Jones, 2008). When it is coupled with brain imaging technologies, self-reporting of pain offers a powerful approach to the understanding and treatment of pain (Jones & Watson, 2007).

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