Patterns of Behavioral Deficits in Rodents Following Brain Injury Across Species, Gender, and Experimental Model

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Introduction

Although rats and mice are the most commonly used lab animals for models of brain injury, few studies have focused on comparative behavioral analyses between the species, and fewer still have assessed gender by species interactions. Rodents, like humans, can perform complex behaviors across a variety of situational demands. However, due in part to their remarkable resiliency, demonstration of consistent and significant behavioral deficits following brain injury can often prove challenging. Variations in testing protocols within and across laboratories, as well as innate differences between rats and mice and their interaction with gender, have muddied the waters even further.

A long history of research has suggested that rats may provide a better model of human behavior than mice, despite their increased cost for experimentation. For decades, rats were the preferred species for studying the effects of brain injury. There are a number of reasons for this preference, including a larger brain (making surgeries somewhat easier) and seemingly more complex behavioral abilities. A review by Wishaw and colleagues [31] concluded that mice present with a simpler, more reflexive repertoire and fewer complex social behaviors. Neuroanatomical differences between rats and mice may account for some of these differences [7, 28]. However, the benefits of mice include lower initial and housing costs, ease of transportation, and the increasing availability of transgenic and knockout mice. For these reasons, many behavioral laboratories, including our own, now test mice more often than rats. Fortunately, behavioral protocols specific to mice are being refined [6]. Furthermore, males are represented in the both the rat and mouse literature to a much larger extent than females. Although avoidance of behavioral variance due to estrous cycles is often cited as a reason to *not* use females, the lack of female representation in the literature presents a broad gap in knowledge, especially in light of the consistent behavioral and neuroanatomical differences observed between human males and females [29].

Our group has tested rodent models of traumatic brain injury to the cortex [1, 3, 8, 9, 14, 16, 23, 24], as well as subcortical injuries such as intracerebral hemorrhage [11–13, 17–19, 21, 26] and hypoxic ischemia encephalopathy [2, 4, 5, 10, 22, 25]. Although these individual studies have provided many insights into the deficits following these injuries and their mechanisms, we have become increasingly interested in comparing the similarities and differences among the behavioral and neuropathological phenotypes induced by different types of brain injury. Because the ultimate goal of these studies is to elucidate the sequelae and mechanisms of brain injury in humans, it is important to determine whether our models' endpoints accurately reflect those observed in humans.

Materials and Methods

For these analyses, water maze and rotarod data from several experiments performed in Loma Linda University's Behavioral Neuroscience Laboratory were combined into a common database consisting of 700 animals (~70 % mice vs. ~30 % rats; ~75 % male vs. ~25 % female). Although the majority of these animals were noninjured or "sham" controls, a subset (~15 %) sustained either traumatic brain injury ("cortical") or intracerebral hemorrhage/ischemia ("subcortical"). Various strains were used for each species, although Sprague-Dawley was the most commonly used rat, and c57Bl/6 was the most commonly used mouse.

The water maze is a test of spatial learning and memory in rodents, requiring the animal to find a slightly submerged platform within a large pool of opaque water. Cumulative distance to the platform (measured 5×/s) was used as the water maze outcome to control for potential differences in swim speed.

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The rotarod test consists of a rotating cylinder placed approximately 30 cm above a tray. The animal is required to walk forward to stay on this cylinder as it rotates to avoid falling. Latency to fall off the rotating cylinder was used as the rotarod outcome. Because the data were gathered over a period of several years and in multiple laboratory environments with diverse research goals, exact implementations of behavioral tests have varied, as have the specific equipment used. Although these differences have increased the variance within the data, the large number of subjects in the database to some extent counteracts these effects. Within- and between-subject ANOVAs were used to analyze the data. For homogeneity of variance and sphericity violations, the appropriate corrections were applied, and significant effects were analyzed with the Scheffé post hoc test.

Results

Overall water maze data suggest that control animals demonstrated an obvious and significant spatial learning curve, in which performance improved with each trial (Fig. 1a). Animals with subcortical injuries and cortical injuries were both significantly impaired. Although both injury groups performed similarly by the end of testing, animals with subcortical injuries started off significantly more impaired. By the end of testing, both types of injury were associated with severe deficits compared with controls. In a comparison of performance by species, both rats and mice started off with similar performance and demonstrated significant learning curves, but the mice reached asymptote earlier than the rats, which continued to improve (Fig. 1b). Across species, males showed slightly, but significantly, improved spatial learning performance compared with females (Fig. 1c). However, when broken down by species and gender (Fig. 1d), it becomes obvious that the effect was driven more by rats, in which males performed significantly better than females. In mice, however, the water maze performance of males and females did not significantly differ.

Overall rotarod performance suggests that cortical injuries produced profound deficits compared with subcortical injuries, which do not differ significantly from controls (Fig. 2a). Similar to the superior cognitive performance of rats in the water maze, rats also demonstrated superior

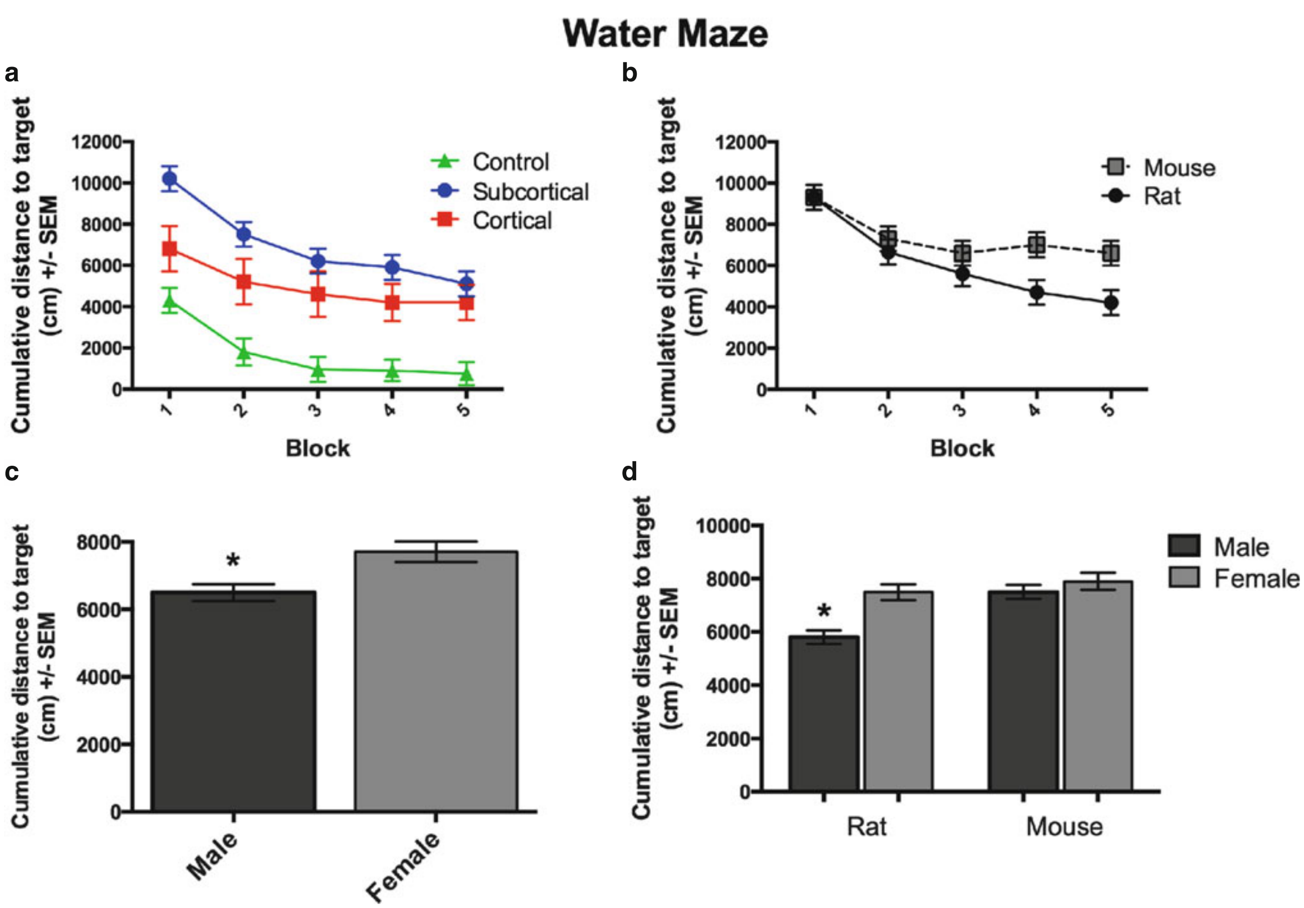

Fig. 1 Spatial learning performance as assessed by the water maze. (**a**) Control animals performed better across the spatial learning trials than cortical animals ($p<.05$), which performed better than subcortical animals ($p<.05$). (**b**) Rats performed better than mice ($p<.05$). (**c**) Males performed better than females ($p<.05$). (**d**) Male rats performed better ($p<.05$) than female rats, male mice, and female mice, which did not differ

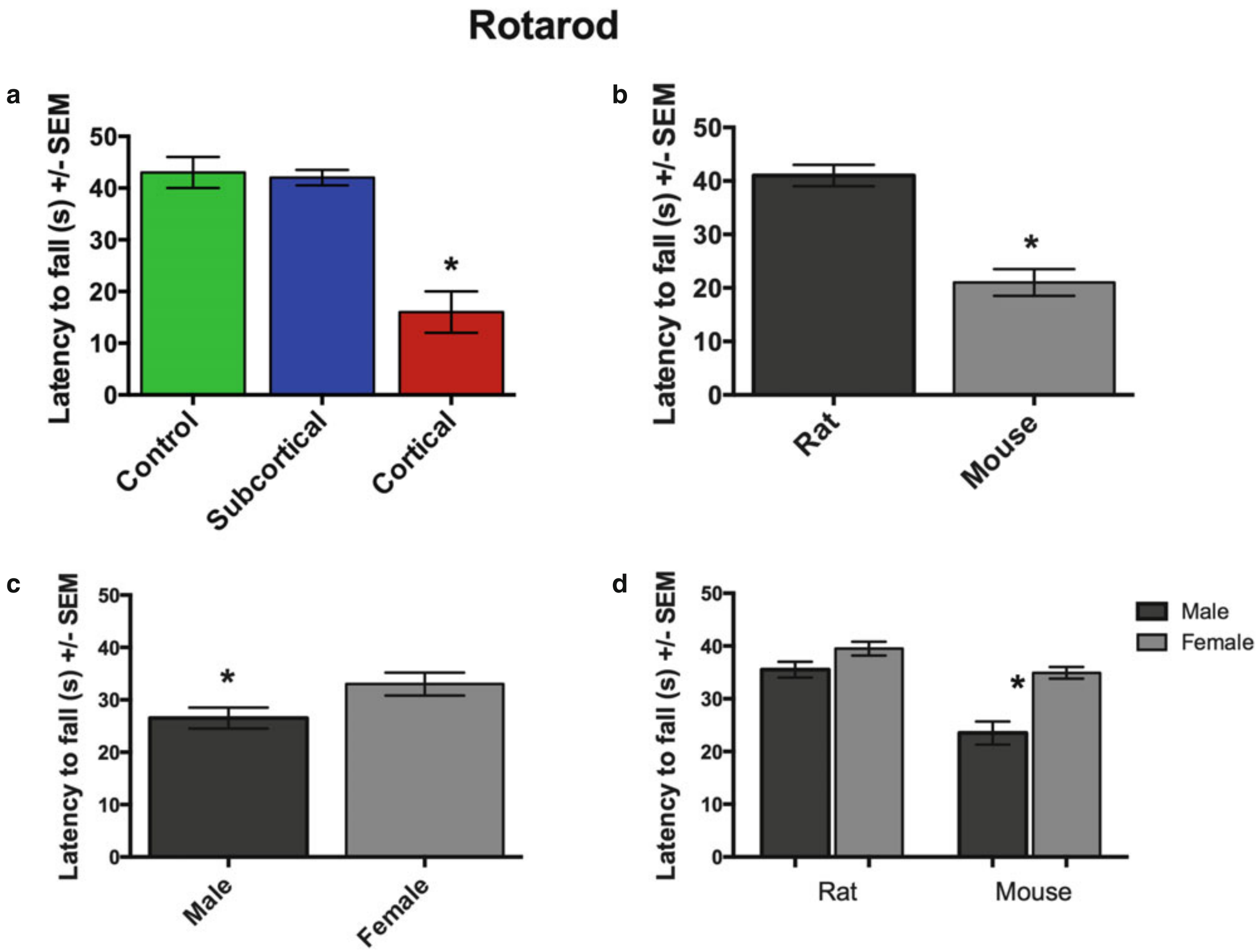

Fig. 2 Sensorimotor balance and coordination performance as assessed by the rotarod. (**a**) Cortical animals performed worse ($p < .05$) than control or subcortical animals, which did not differ. (**b**) Mice performed worse than rats ($p < .05$). (**c**) Males performed worse than females ($p < .05$). (**d**) Male mice performed worse ($p < .05$) than female mice, male rats, and female rats, which did not differ

sensorimotor coordination and balance on the rotarod by staying on the rotating cylinder significantly longer than mice (Fig. 2b). Across species, females showed slightly, but significantly, improved performance compared with males (Fig. 2c). However, when broken down by species and gender (Fig. 2d), it becomes obvious that the effect was driven more by mice, in which males performed significantly worse than females. In rats, however, males performed only slightly worse than females.

Discussion

These findings extend the existing body of literature by making comparisons among species, gender, and injury model across cognitive and sensorimotor behavioral domains. We have also shown that cortical injuries, such as those induced by a traumatic brain injury, were associated with profound sensorimotor and cognitive deficits. In contrast, subcortical injuries, such as those induced by intracerebral hemorrhage, hypoxic-ischemia, or both, were associated with even more profound cognitive deficits in the absence of significant sensorimotor deficits. The observed patterns of performance indicate the similarities of the broad pattern of neurocognitive deficits associated with these rodent brain injury models and those observed following human brain injury.

Likewise, we observed subtle gender differences that proved to be species-dependent. As often reported in humans, males performed subtly, but significantly, better than females on a task of spatial learning. However, this phenomenon was only observed in rats, and mice of both genders performed more like female rats in the water maze. The opposite pattern was observed on a test of sensorimotor coordination and balance. In general, males performed subtly, but significantly, worse than females on the rotarod. However, this phenomenon was most evident in mice, and rats of both genders performed similarly to female mice. Although many studies from our laboratory have not found statistically significant differences in gender, these results suggest that consistent, but subtle, differences exist. Indeed, a meta-analysis of rodent sex differences by Jonasson [15] concluded that there was evidence for gender differences in spatial learning, despite the fact that the majority of reviewed studies reported no significant differences.

Finally, we observed that, in general, rats performed better across both cognitive and sensorimotor behavioral domains. However, as noted for spatial learning in the water maze, this was only true for male rats. For sensorimotor coordination and balance on the rotarod, only male mice performed worse than rats. These results at least partly corroborate those reported in the literature. When Wishaw and Tomie [32] used the water maze to assess spatial learning in rats and mice, rats performed significantly better. Moreover, the rats demonstrated improved problem-solving strategies over time relative to mice, indicating that they were learning to learn, whereas mice showed no such problem-solving strategy improvement. Even though strain differences have been observed in both rats [20] and mice [27] due to varying swim speeds and visual acuity, rats' ability to conduct an organized search with high accuracy suggests that they are better problem solvers in the water. Even though it has been suggested that the Barnes maze, a dry land alternative to the water maze, may be more appropriate for mice, mice in our hands have performed similarly on both the Barnes maze and the water maze. Additionally, similar to our rotarod results, rats have demonstrated a greater ability to learn complex and coordinated asymmetrical motor behavior than mice in a test of reaching behavior and environmental manipulation [30].

Significant differences in task performance and trends in performance could indicate that particular species and gender combinations could provide better models for humans, or reveal the most important controls to consider, depending on the behavior of interest. Notably, gender differences were present, but were not the same for rats and mice. Mice demonstrated more pronounced gender differences in motor ability than rats, whereas rats demonstrated more pronounced gender differences in spatial learning. These findings have implications for study design. The extensive time and budget resources required for administration of a comprehensive battery requires that researchers use the most efficient design. Strategically designed batteries could identify initial behavioral outcomes and indicate the need for future behavioral testing while consuming the fewest resources. Depending on the nature of the injury and the deficits produced, the economic benefits of using mice over rats may be offset by the larger number of animals required to attain sufficient statistical power.

In summary, this study showed that the cognitive and sensorimotor abilities of rodents differ according to species, gender, and type of injury. This study also characterized distinct behavioral profiles for animals with cortical and subcortical brain injury models that resemble injury profiles in humans. Additional covariates, such as edema and lesion size, may further clarify these phenotypes. Overall, we provide evidence that abbreviated test batteries may be specifically designed to test deficits depending on the species, gender, and model.

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Conflict of Interest The authors declare that they have no conflict of interest.

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