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Disrupted Sleep and Delayed Recovery from Chronic Peripheral Neuropathy Are Distinct Phenotypes in a Rat Model of Metabolic Syndrome

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ABSTRACT

Background: Sleep apnea, hypertension, atherosclerosis, and obesity are features of metabolic syndrome associated with decreased restorative sleep and increased pain. These traits are relevant for anesthesiology because they confer increased risks of a negative anesthetic outcome. This study tested the one-tailed hypothesis that rats bred for low intrinsic aerobic capacity have enhanced nociception and disordered sleep.

Methods: Rats were developed from a breeding strategy that selected for low aerobic capacity runners (LCR) and high aerobic capacity runners (HCR). Four phenotypes were quantified. Rats underwent von Frey sensory testing (n = 12), thermal nociceptive testing (n = 12), electrographic recordings of sleep and wakefulness (n = 16), and thermal nociceptive testing (n = 14) before and for 6 weeks after a unilateral chronic neuropathy of the sciatic nerve.

Results: Paw withdrawal latency to a thermal nociceptive stimulus was significantly ($P < 0.01$) lower in LCR than HCR rats. There were also significant differences in sleep, with LCR rats spending significantly ($P < 0.01$) more time awake (18%) and less time in nonrapid eye movement sleep (−19%) than HCR rats. Nonrapid eye movement sleep episodes were of shorter duration (−34%) in LCR than HCR rats. Rapid eye movement sleep of LCR rats was significantly more fragmented than rapid eye movement sleep of HCR rats. LCR rats required 2 weeks longer than HCR rats to recover from peripheral neuropathy.

Conclusions: Rodents with low aerobic capacity exhibit features homologous to human metabolic syndrome. This rodent model offers a novel tool for characterizing the mechanisms through which low aerobic function and obesity might confer increased risks for anesthesia.

What We Already Know about This Topic

- Metabolic syndrome is increasingly common in surgical patients and carries increased perioperative risks.

What This Article Tells Us That Is New

- A breeding strategy was used to create rats with low intrinsic aerobic capacity that provide a model of metabolic syndrome.
- These rats have enhanced nociception and disrupted sleep, and show promise for studies of how obesity and diminished aerobic function increase anesthetic and perioperative risks.

METABOLIC syndrome and associated comorbidities have special relevance for anesthesiology.[1–3] Central obesity is a feature of metabolic syndrome that confers an increased risk of type II diabetes,[4] and the combination of obesity and diabetes is associated with increased perioperative risks.[5–7] Obesity increases risk for aspiration during induction,[8] decreases perioperative tissue oxygenation,[9] is a predictor for difficult mask ventilation,[10] and is a risk factor for anesthesia-related maternal mortality.[11] Metabolic syndrome also is associated with poor sleep,[12–20] and disrupted sleep can contribute to metabolic syndrome.[13,17–19,21,22] Metabolic syndrome increases risk for chronic neuropathic pain,[23] and sleep disruption enhances pain perception.[24–28]

The complexity and bidirectional nature of the foregoing pathologies emphasize the need for animal models that can help elucidate mechanisms underlying interactions among metabolic syndrome, sleep, and pain. Two broad observa-

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tions suggest that aerobic capacity and its attendant subordinate phenotypes are critical in the divide between health and disease, and motivated the development of rat models of disease risk. First, clinical studies reveal a strong statistical relationship between dysfunctional oxygen metabolism and all-cause morbidity and mortality. Indeed, for healthy individuals as well as those with cardiovascular disorders, low exercise capacity is a stronger predictor of decreased survival compared with other conventional risk factors such as body mass index, smoking, hypertension, dyslipidemia, or diabetes.[29] Second, exercise is an effective prescription for a surprisingly wide range of chronic diseases.[30]

These ideas formed the basis for speculating that experimental models that divide for health and disease could be created by selection for low and high intrinsic aerobic endurance running capacity. Starting from a genetically heterogeneous population of rats developed at the National Institutes of Health (Bethesda, Maryland), a divergent (two-way) artificial selection process was used to generate lines of low aerobic capacity runners (LCR) and high aerobic capacity runners (HCR).[31] At 10 generations of selection, the two lines diverged widely for expected running capacity and hypothesized differences for health risks, including metabolic syndrome.[32]

Development of LCR and HCR lines generated a unique model system for dissection of aerobic endurance capacity and its correlated health-related phenotypes. The major hypothesis is that functional alleles at multiple interacting loci that affect intrinsic aerobic capacity have been enriched or fixed differentially between the LCR and HCR lines. It is critical that the models are maintained as genetically heterogeneous lines by using a mating paradigm that minimizes inbreeding.[31] Compared with inbred strains (in which essentially all loci have been taken to fixation), outbred selected lines maintain genetic complexity that allows combinations of allelic variants at multiple interacting loci to be enriched by selection pressure.[33] As a result, the LCR-HCR model is better suited to discover epistatic interactions, modifier genes, and synergistic actions.[34] It is noteworthy that the concurrent breeding of LCR and HCR rats at every generation allows the lines to serve as reciprocal controls for unknown environmental changes.

The present study tested the hypothesis that LCR rats, in addition to demonstrating a predisposition for metabolic syndrome,[32,35] also have altered nociception and disrupted sleep. The results are consistent with the notion that low intrinsic aerobic capacity and attendant correlated traits such as obesity can at least partly underlie pathology associated with altered nociception and disrupted sleep.

Materials and Methods

Animals

Experiments were conducted in accordance with the Policy on Humane Care and Use of Laboratory Animals established by the National Institutes of Health (publication 80–23). All procedures were reviewed and approved by the University of Michigan Committee on Use and Care of Animals (Ann Arbor). This study used a recently developed rat model selectively bred as LCR and HCR. These rats were derived from a genetically heterogeneous founder population developed at the National Institutes of Health.[36] As described in detail previously,[31] a large-scale rotational breeding strategy was started in 1996 to develop lines of rats that differ for intrinsic (*i.e.*, untrained) aerobic capacity and retain wide heterogeneity. Each rat in the founder population (96 males, 96 females) was tested for maximal endurance running capacity on a speed-ramped treadmill. This test was patterned after clinical treadmill running tests and provided a reliable estimate of aerobic capacity (VO_2 max) to segregate rats into the lowest and highest capacity runners. Twenty-six mating pairs were selected to create 13 base families of LCR and 13 base families of HCR. Offspring arising from these LCR and HCR breeder animals were tested at 11 weeks of age for intrinsic running capacity. The highest performing male and female in each HCR family and the lowest performing male and female from each LCR family served as parents for each subsequent generation. After 11 generations of selective breeding, LCR and HCR rats differed by almost 350% for running capacity endurance with the LCR rats exhibiting several features consistent with metabolic syndrome.[32]

Rats used in the present study were males from generations 20 and greater. Differences in body weight between the two lines are illustrated by figures 1A and 1B. Typical weights ranged from approximately 380 g (HCR) to 550 g (LCR). The difference in aerobic phenotype of distance run-to-exhaustion by HCR and LCR rats has been quantified for generation 26 and is illustrated in figure 1C. Two key points are that (1) these rats were never exercise-trained, and (2) the breeding procedure used made it possible for the present study to evaluate the impact of genetically segregated intrinsic capacity for oxygen metabolism on nociception and sleep. Table 1 summarizes previously documented physiologic, behavioral, and metabolic differences between HCR and LCR rats.

Experiment 1: Quantifying Sleep

There is a convergence of evidence that sleep neurobiology can help elucidate some of the mechanisms by which anesthetics cause a loss of wakefulness.[37–41] Therefore, the goal of the first experiment was to phenotype states of sleep and wakefulness as a prelude to future studies designed to characterize responses of LCR and HCR rats to intravenous and volatile anesthetics. The procedures used to study sleep in rats have been described in detail.[42,43] In brief, eight male LCR rats and eight male HCR rats were anesthetized with 3% isoflurane in 100% oxygen (O_2), delivered at a flow rate of 1 l/min. Delivered isoflurane concentration was measured by spectrometry (Cardiocap™/5; Datex-Ohmeda, Louisville, CO). Once anesthetized, each rat was placed in a Kopf Model 962 small animal stereotaxic frame with a Model 906 rat anesthesia mask (David Kopf Instruments, Tujunga, CA), and delivered isoflurane concentration was decreased to 1.5%. Core body temperature was maintained at 37°C

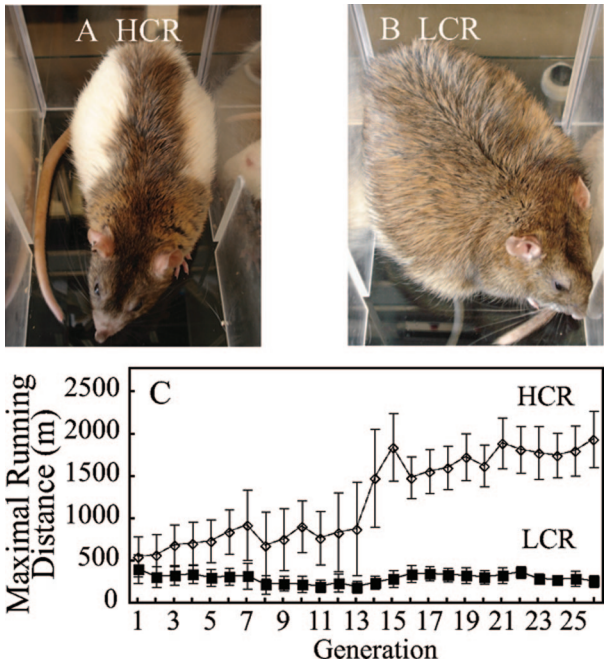

Fig. 1. High intrinsic aerobic capacity running (HCR) and low intrinsic aerobic capacity running (LCR) rats differ in body weight and maximal running distance. Photographs of representative animals show that HCR rats (*A*) were lean and weighed approximately 30% less than LCR rats (*B*). Distance run-to-exhaustion (*C*) for HCR and LCR rats plotted as a function of different breeding generations. Selective breeding of generations 1–26 required approximately 14 yr. Aerobic performance shown by this graph represents intrinsic (*i.e.*, untrained) aerobic ability.

throughout the surgical procedure with the use of a recirculating heat pump (Gaymar Industries, Inc., Orchard Park, NY). A midline scalp incision was made and electrodes were implanted for electroencephalogram and electromyogram recording. The cortical electroencephalogram electrodes (8IE36320SPCE; Plastics One, Roanoke, VA) were screwed into the cranium at three sites relative to bregma: 2.0 mm posterior, 1.3 mm lateral; 2.0 mm posterior, −1.5 mm lateral; and 1.0 mm anterior, 1.5 mm lateral. Two electromyogram electrodes were constructed from AS632 biomed wire (Cooner Wire, Inc., Chatsworth, CA) and were placed into the dorsal neck muscles. Another electrode was placed subcutaneously near the dorsal aspect of the skull and served as an indifferent electrode. Leads from all six electrodes were placed together in a six-pin multichannel electrode pedestal (MS363, Plastics One) that was secured to the skull with three anchor screws and dental acrylic (Jet Acrylic Self Curing Resin and Liquid; Lang Dental Manufacturing Company, Inc., Wheeling, IL).

After surgery, rats were allowed to recover for 7–10 days while habituating to the sleep recording chambers. Habituation consisted of placing the rats in the recording chambers and connecting the electrode pedestal on the skull to a six-channel cable (363-441/6 80CM 6TCM, Plastics One) that led to amplifiers and a computer. Housing and sleep recordings occurred in a 12:12 light-dark cycle with transitions occurring at 8:00 AM and 8:00 PM. States of sleep and wakefulness were recorded across 24 h in two continuous 12-h segments that coincided with light and dark cycle start times. The electrographic signals were amplified, filtered (electroencephalogram, 0.3–30 Hz; electromyogram, 10–100 Hz), and recorded at 128 Hz. After habituation, states of sleep and wakefulness were recorded for 24 h. For each 24-h digital recording, 10-s bins were scored as wakefulness, rapid eye movement (REM) sleep, or non-REM (NREM) sleep. During recording sessions, the rats could move freely and had *ad libitum* access to food and water.

Experiment 2: Von Frey Testing

A second group of LCR (n = 6) and HCR (n = 6) rats was used to phenotype mechanoreceptor thresholds by measuring paw withdrawal away from 2, 8, and 15 g of pressure caused by von Frey hairs applied to the bottom of a hind paw (Touch Test Sensory Evaluator Kit; Stoelting Co., Wood Dale, IL). As described previously,[44,45] these nylon hairs were slowly pushed against the skin until the hair bent. Each of the three hairs was applied five times in ascending order. Paw withdrawal was recorded as a positive response and the percentage response to each von Frey hair was tabulated and averaged for each animal.

Experiment 3: Thermal Nociception

As described previously,[46] rats were conditioned daily to being placed in a chamber for nociceptive testing (model 336T;

Table 1. Physiological, Metabolic, and Behavioral Characteristics of High *versus* Low Aerobic Capacity Runner Rats

High Aerobic Capacity	Low Aerobic Capacity
Run to exhaustion, ~70 min	Run to exhaustion, ~13 min
More spontaneous cage activity[90]	Displays features of metabolic syndrome[32]
High resting and maximal oxygen consumption[88]	Marked weight gain and increased insulin resistance on a high fat diet[35]
Resistant to weight gain and altered insulin resistance on a high fat diet[35]	Manifest spontaneous hepatic steatosis[90]
Skeletal muscle	Susceptible to ischemia-mediated ventricular tachyarrhythmias[91]
36% more capillaries[89]	
Increased mitochondrial oxidative proteins[32]	
Increased lifespan (~6 months)[32]	

IITC Life Science, Inc., Woodland Hills, CA). The Hargreaves device made it possible to quantify the latency in seconds to paw withdrawal (PWL) away from onset a light beam focused on the hind paw.[47] On the day of testing, animals were placed in the Hargreaves chamber and allowed to habituate for 60 min before obtaining measures of PWL. The light beam and an electronic timer were activated simultaneously. When the rat lifted its hind paw, the heat source and timer were stopped and PWL was recorded.[46,48,49] Five measurements were obtained and averaged during 5 min of testing.

Experiment 4: Quantifying Recovery from Chronic Peripheral Nerve Injury

A unilateral chronic peripheral mononeuropathy[50] of the sciatic nerve was created in adult male LCR (n = 7) and HCR (n = 7) rats. Before surgery, each rat was anesthetized with 3% isoflurane until breathing rate decreased to 60 breaths per min. After loss of wakefulness, the rat was fitted with a Kopf Model 906 rat anesthesia mask and isoflurane concentration was lowered to 1.5% for the remainder of the procedure. Delivered isoflurane concentration and core body temperature were measured. The sciatic nerve was accessed *via* hind limb incision followed by blunt dissection of the biceps femoris. At a level proximal to its trifurcation, 5–8 mm of nerve were separated from tissue with minimal damage to surrounding muscle and tissue.[50] Three polyglycolic acid sutures were placed around the sciatic nerve at 1-mm intervals, loosely enough to prevent complete ligation but tightly enough to induce a leg twitch response.[50] The wound margin was sutured closed and rats were allowed 6 days to recover from surgery.

Thermal nociceptive testing was performed before and after the chronic constriction injury. A beam of light, which served as a noxious thermal stimulus, was directed through a glass floor and onto the plantar aspect of the hind paw. This light remained on until the rat withdrew its paw from the thermal stimulus. PWL was defined as the time between commencement of the light beam and withdrawal of the hind paw.[47] PWL was measured for all rats the day before sciatic nerve ligation and on days 7, 14, 21, 28, 35, and 42 after nerve ligation. During this 6-week testing period, rats were conditioned in the testing chamber for 2 h daily. At the beginning of each testing day, rats were habituated to the chambers for 1 h before five PWL measurements were obtained with the Hargreaves method applied to the hind paw of the operated and nonoperated limb. To prevent tissue damage during nociceptive testing, the thermal light stimulus was set to terminate automatically (cut-off time) if there was no paw withdrawal after 18 s.

Statistical Analyses

This study tested the one-tailed hypothesis that rats bred for low intrinsic aerobic capacity have enhanced nociception and disordered sleep. The 24-h recordings of sleep and wakefulness were scored for 138,240 epochs, each 10 s in duration. To avoid inflated degrees of freedom, data were averaged for each of the 16 rats. Dependent measures included the percentage of recording time spent in wakefulness, NREM sleep, and REM sleep; episode duration and number of episodes for each of the three states; and number of transitions between states. The data for percent of time spent in each state were normally distributed and fit the assumptions of two-way analysis of variance for repeated measures. The data describing recovery from chronic constriction nerve injury as a function of time were also analyzed using two-way analysis of variance for repeated measures. *Post hoc* comparisons were made using Mann-Whitney and Bonferroni statistics. The von Frey data were not normally distributed and were analyzed using the Mann-Whitney statistic. Software used for statistical analyses included SAS (version 9.2; SAS Institute, Inc., Cary, NC), GB-Stat (Dynamic Microsystems, Inc., Silver Spring, MD), and Prism (version 5; GraphPad Software, Inc., La Jolla, CA).

Poincaré analyses are particularly useful for quantitatively evaluating the periodic nature of bistable rhythms such as the cardiac cycle,[51] breathing,[52,53] and sleep.[54] Poincaré analyses were used in the present study to compare LCR and HCR animals for their ability to maintain stable episodes of REM sleep. Poincaré analysis made it possible to quantify two indices of variability. Short-term variability (SD1) expressed the variability in time from one REM sleep episode to the next REM sleep episode. Long-term variability (SD2) quantified the overall variability in duration of REM sleep epochs across the 24-h recording. Calculation of SD1 values for LCR and HCR rats was achieved with a four-step process: (1) duration in seconds of each REM sleep interval was plotted y relative to x, representing the duration of the previous REM sleep interval. (2) After all REM sleep epochs were plotted, the line of identity ($x = y$) was calculated and added to the Poincaré graph. (3) The Pythagorean Theorem was used to calculate the perpendicular distance between each data point and the line of identity. (4) The standard deviation of these raw distances was calculated as the SD1 value for each LCR and HCR rat, and these values were averaged across all LCR and HCR rats, respectively. SD2 values were obtained using the first step noted above and calculating the distance of each point from a line perpendicular to the line $y = x$ and running through the mean duration of REM sleep intervals (*i.e.*, centroid of data points). As a final step, individual animal means of SD1 and SD2 for each LCR and HCR rat were used to evaluate rat strain differences by t test. For all inferential statistics a P value of less than 0.05 was considered significant.

Results

The Time Spent in States of Sleep and Wakefulness Varied as a Function of Aerobic Capacity

Figure 2 illustrates the temporal distribution of sleep and waking states recorded continuously during a 24-h period for one representative LCR rat and one representative HCR rat. Figure 3 summarizes the average of eight 24-h recordings for

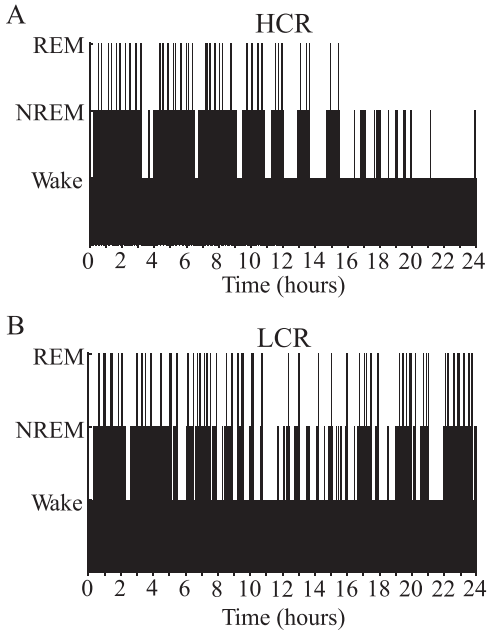

Fig. 2. States of wakefulness (Wake), nonrapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep were recorded electrographically for 24 h (abscissa). Plots show sleep architecture of one representative high aerobic capacity runner (HCR) rat (*A*) and one representative low aerobic capacity runner (LCR) rat (*B*). The height of each bar identifies the behavioral state and the width of the bars codes for duration of time spent in each state. Hours 0 to 12 correspond to the light phase of the 12:12 light-dark cycle (rat subjective night). The recording interval from12:00 to 24:00 h corresponds to the dark phase (subjective day) of the light-dark cycle. Note that sleep in the HCR rat was consolidated during the light phase, whereas the sleep of the LCR rat was distributed throughout the 24-h period. Figures 3–5 quantitatively characterize strain-specific changes in sleep architecture.

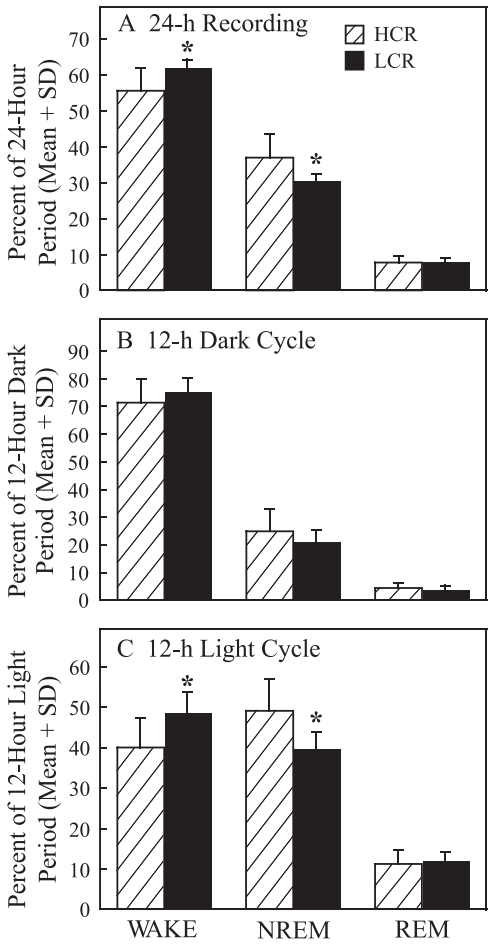

Fig. 3. Low aerobic capacity runners (LCR) spent more time awake and less time in nonrapid eye movement (NREM) sleep than high aerobic capacity runners (HCR). Asterisks indicate statistically significant differences between HCR and LCR rats. There were no differences in the rapid eye movement (REM) phase of sleep. (*A*) Differences in sleep as a function of rat strain were apparent across 24 h of continuous sleep/wake recordings. (*B*) There was no significant difference in the amount of time spent asleep or awake during the dark phase of the light-dark cycle. (*C*) The sleep/wake differences between LCR and HCR rats resulted from differences in arousal state distribution during the light phase, when these nocturnal animals normally sleep. Data summarize eight 24-h recordings from HCR rats and eight 24-h recordings from LCR rats.

each strain and quantifies strain-specific differences in sleep architecture. During the 24-h recordings, LCR rats spent significantly ($P < 0.009$) more time in wakefulness and significantly ($P < 0.01$) less time in NREM sleep than HCR rats (fig. 3A). These differences were not apparent in the dark portion of the light-dark cycle (fig. 3B) and resulted predominantly from significant differences in wakefulness ($P < 0.007$) and NREM sleep ($P < 0.005$) that occurred during the light phase of recording (fig. 3C). LCR and HCR rats did not differ in the percentage of recording time spent in REM sleep (fig. 3).

Poincaré Analyses Revealed Differences in REM Sleep as a Function of Aerobic Capacity

Figure 4 summarizes the variability in duration of REM sleep episodes characteristic of LCR and HCR animals. Comparison of the Poincaré distributions for HCR (fig. 4A) and LCR (fig. 4B) rats indicates differences in the variability of REM sleep duration. Figure 4C illustrates the significantly ($P < 0.05$) greater SD2 in REM-sleep episode duration characteristic of LCR rats. Short-term variability (SD1) in a Poincaré analysis quantifies the duration of one REM sleep episode (REM_i) relative to the previous REM sleep episode (REM_i-_1). The differences in short-term variability between LCR and HCR rats were not significant. Long-term variability (SD2) quantifies the variability in REM-sleep episode duration as a function of rat strain. Figure 4C shows that SD2 was greater for LCR than HCR rats. Thus, LCR animals could initiate but not maintain stable REM sleep episodes. Comparing REM sleep data in figures 2–4 demonstrates how Poincaré analyses made it possible to quantify differences in the temporal organization of REM sleep.

The results of the Poincaré analyses encouraged a more detailed comparison of the temporal organization of sleep-wake states, and figure 5 summarizes those results. Averaging across 24 h, LCR animals had significantly ($P < 0.001$) more

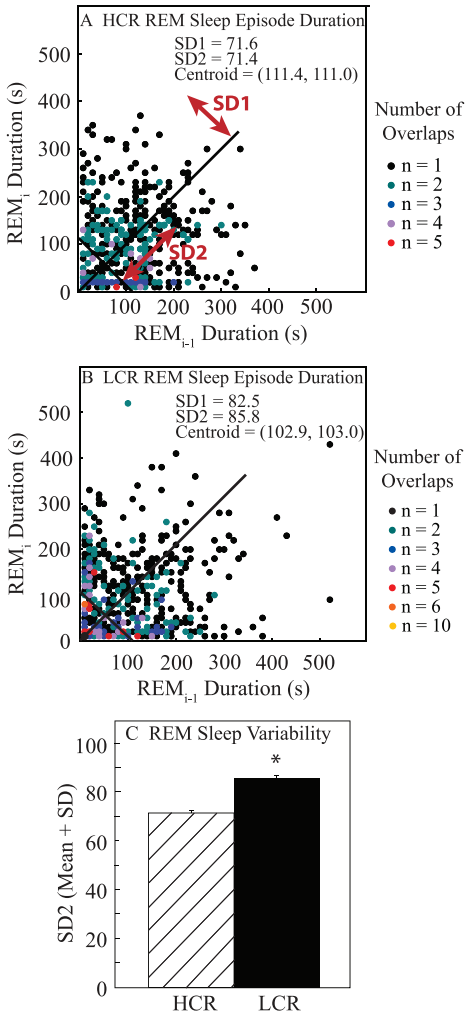

Fig. 4. Poincaré analyses revealed differences in rapid eye movement (REM) sleep duration as a function of aerobic capacity. (*A* and *B*) Each point represents the duration in seconds of one REM sleep episode (y-axis REM_i) relative to the duration in seconds of the previous episode of REM sleep (x-axis REM_i-_1). These plots summarize 463 REM sleep episodes for high aerobic capacity rats (HCR) and 450 REM sleep episodes for low aerobic capacity (LCR) rats. (*A*) illustrates key features used to interpret the Poincaré plot. Short-term variability (SD1) is the SD for the dispersion of points relative to the line of identity (x = y). Short-term variability characterizes the variability in REM sleep duration from one REM sleep episode to the next. The centroid indicates the average duration of REM sleep episodes and is indicated at the point where the perpendicular line intersects the line of identity. The dispersion of points orthogonally to the line of identity was not significantly different in A and B, indicating no significant difference in short-term variability between HCR and LCR strains. Long-term variability (SD2) represents the dispersion of data points relative to the line passing through the centroid. Long-term variability conveys the variability in REM sleep duration as a result of aerobic capacity. The color codes at right indicate a larger number of overlapping data points in B than in A. (*C*) Quantitative comparison of long-term variability revealed that low aerobic capacity rats had significantly more long-term variability in REM sleep duration than HCR rats. Thus, REM sleep was initiated more often by LCR rats than HCR rats and could not be maintained by LCR rats. Asterisk indicates a statistically significant difference between HCR and LCR rats.

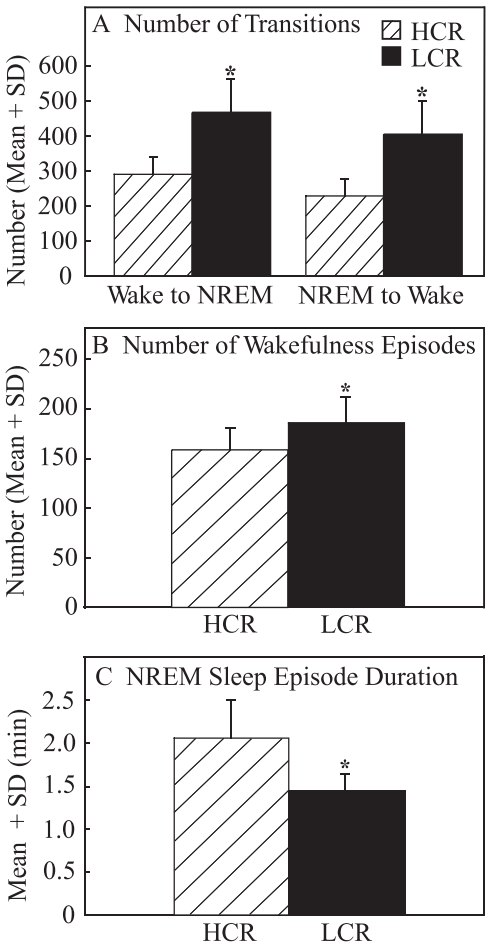

Fig. 5. The temporal organization of wakefulness and nonrapid eye movement (NREM) sleep varied as a function of aerobic capacity. Asterisks indicate statistically significant differences between high capacity runners (HCR) and low capacity runners (LCR). (*A*) The number of transitions between NREM sleep and wakefulness (Wake) was significantly greater in LCR rats than in HCR rats. (*B*) Across the 24-h sleep recordings LCR animals also had significantly more wakefulness episodes. (*C*) Averaging the duration of the NREM sleep episodes revealed a shorter duration in LCR than HCR rats.

transitions from wakefulness to NREM sleep and significantly ($P < 0.001$) more transitions from NREM sleep to wakefulness than HCR rats (fig. 5A). Consistent with results presented in the figure 1, across the 24-h recording, LCR rats had a significantly ($P < 0.04$) greater number of wakefulness episodes than HCR rats (fig. 5B). When LCR rats had NREM sleep, the average duration of each NREM sleep episode was significantly ($P < 0.002$) shorter than that of HCR rats (fig. 5C).

There was no difference between LCR and HCR rats in mechanosensory response as measured by von Frey testing (fig. 6A). Power calculation indicated that 61 rats would be needed to demonstrate a difference in von Frey response at a *P* value lower than 0.05. As described previously,[46] LCR rats revealed a modest but significantly shorter PWL in response to a thermal nociceptive stimulus (fig. 6B). Figure 6C shows the time course

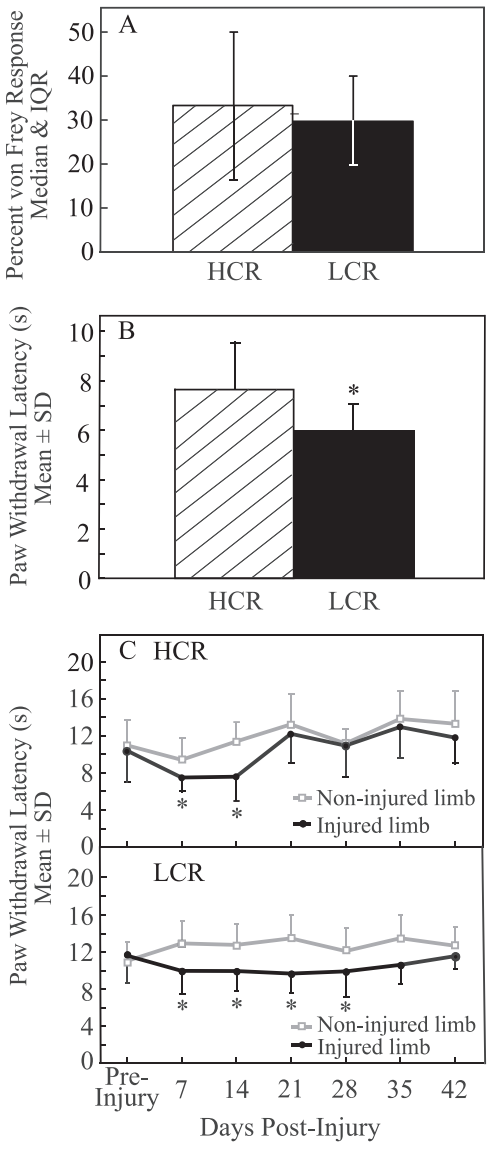

Fig. 6. Sensory-motor and nociceptive responses in HCR and LCR rats. (*A*) Median and interquartile range (IQR) show no difference in paw withdrawal latency in response to von Frey hair stimulation between high capacity runners (HCR, n = 6) and low capacity runners (LCR, n = 6). (*B*) In a separate group of HCR (n = 6) and LCR (n = 6) rats Hargreaves testing was performed with no other manipulation. These measures of baseline nociception revealed LCR animals to have significantly shorter paw withdrawal latency than the HCR rats.[46] (*C*) HCR and LCR animals differed significantly in the time needed to recover from chronic constriction injury of the sciatic nerve, a model of a chronic pain. Low intrinsic aerobic capacity (LCR) rats (n = 7) revealed a delayed recovery from peripheral neuropathy compared with high intrinsic aerobic capacity (HCR) rats (n = 7). Asterisks indicate statistically significant differences at specific time points after experimentally induced injury of the sciatic nerve. For LCR and HCR rats at 7 and 14 days after sciatic nerve injury, there was a significantly shorter latency for paw withdrawal of the injured hind limb. The LCR rats showed hyperalgesia for a month, whereas the HCR rats recovered in 2 weeks.

of PWL measured in LCR and HCR rats 1 week before and 42 days after chronic constriction injury of one sciatic nerve.[50] Each of the seven points in figure 6C summarizes mean ± SD PWL in response to a thermal stimulus. Before the peripheral neuropathy (preinjury) there was no statistically significant difference in PWL between LCR and HCR animals. For LCR rats (fig. 6C), two-way analysis of variance for repeated measures revealed a significant effect of peripheral neuropathy on PWL. *Post hoc* tests revealed significantly ($P < 0.05$) enhanced nociception in LCR rats on days 7, 14, 21, and 28 after peripheral neuropathy. Measures of PWL in HCR rats (fig. 6C) revealed significantly ($P < 0.05$) enhanced nociception on days 7 and 14 after chronic constriction injury of the sciatic nerve.

Discussion

In 2000, the National Task Force on the Prevention and Treatment of Obesity reported delayed recognition of the fact that obesity is a significant health risk.[55] Although relevance of obesity for anesthesiology was noted in 1975,[56] only recently has the interaction among obesity, sleep, and pain become apparent.[3,5,37,57] Obesity has replaced smoking as a major disease burden[58] and projection data indicate that by 2020, half of all Americans may be obese.[59] The results are discussed relative to emerging evidence that obesity is associated with disordered sleep, potentially causing hyperalgesia and increase opioid requirement for effective pain management.

Aerobic Fitness, Metabolic Syndrome, and Sleep

Data from LCR rats are consistent with human data documenting a relationship among metabolic syndrome, sleep, and nociception. For example, humans with poor physical fitness and a high body mass index exhibit disrupted and diminished sleep compared with those who maintain a normal body mass index.[18,20,60] Humans with metabolic syndrome and type II diabetes spend more time in bed and less time asleep.[12,13,16,17]

Human data also support the view that reduced sleep leads to increased body mass and decreased fitness by way of increasing cortisol, interleukin-6, and ghrelin, and decreasing leptin.[17–19] Thus, shortened sleep has been associated with decreased fitness. The present results show for the first time that diminished aerobic fitness in LCR rats is characterized by disordered sleep. In addition, low intrinsic aerobic capacity was associated with decreased NREM sleep time and increased time awake (figs. 2 and 3). Sleep disruption in rats with low aerobic capacity was also characterized by greater variability in REM sleep duration (fig. 4) and more transitions between wakefulness and NREM sleep (fig. 5) compared with rats bred for high intrinsic aerobic capacity. The decreased amount of sleep and inability to maintain sleep characterizing this rodent model of metabolic syndrome[31,32,61] resemble features of human insomnia. In support of the potential clinical relevance of this rodent model, the disordered sleep and short sleep duration characteristic of human insomnia are risk factors for type II diabetes.[62] The present results encourage future studies designed to obtain long-term recordings of sleep and wakefulness from LCR and HCR rats.

Aerobic Fitness, Disordered Sleep, and Nociception

Obesity and insulin resistance,[63–65] specific features of metabolic syndrome, are associated with painful, idiopathic neuropathy,[23] enhanced nociception,[66,67] and disordered sleep.[68–71] Although data clearly document an association between metabolic syndrome and pain, no previous studies have characterized the effect of metabolic syndrome on the resolution of experimentally imposed, chronic pain. Compared with HCR rats, the LCR phenotype revealed a delayed recovery from chronic peripheral mononeuropathy (fig. 6C). Results presented in figure 6C, and the fact that LCR and HCR rats have been bred for the intrinsic (*i.e.,* untrained) component of aerobic capacity, imply that genetic and metabolic factors altered the resolution of the neuropathy. This implication fits with the finding that human obesity is associated with increased hospital admissions and longer hospital stays.[72] The disordered sleep characteristic of LCR rats may also contribute to hyperalgesia. In normal, pain-free humans, sleep loss causes hyperalgesia[25,28] and nonrestorative sleep affects pain management.[73] Opioids disrupt NREM sleep and inhibit the REM phase of sleep.[38] REM sleep disruption has consistently been shown to diminish the analgesic properties of opioids.[74]

Models, Limitations, and Future Directions

There is good precedent for animal models contributing to clinical anesthesiology. Rodent studies have provided the background for spinal drug administration now routine in clinical care,[75] porcine models helped elucidate the mechanisms of malignant hyperthermia,[76] and unique features of goat brain vasculature helped differentiate brain *versus* spinal cord sites of anesthetic action.[77] All models have a limited domain of applicability and the strengths and limitations of LCR and HCR rats have been described in detail.[61] A known limitation of sensory threshold testing is that the data are characterized by a high degree of variability.[78,79] This variability may contribute to the lack of rat strain–specific differences in response to von Frey testing (fig. 6A), as well as differences between LCR and HCR rats during acute nociceptive testing (fig. 6B), but not during preinjury nociceptive testing (fig. 6C). Likewise, the complexity of animal models of nociception is recognized[80] and future nociceptive testing should include additional pain models and sensory modalities.

The polygenic nature of complex diseases emphasizes the advantages of models created by the selective breeding for naturally occurring traits over transgenic models that manipulate a single gene or gene product.[61] This advantage is particularly clear for efforts to model hypertension, metabolic syndrome, and disordered sleep, all of which complicate anesthesia care. The potential for LCR and HCR rats to provide a unique resource for anesthesiology research is supported by evidence emphasizing the biologic centrality of oxygen uptake and delivery[61] and the special relevance of oxygen for anesthesiology.[81]

The present findings suggest homology between obese human and rat for the traits of sleep fragmentation and nociception, and support the interpretation that LCR rats develop some features of metabolic syndrome.[32] Sleep apnea is the second most common sleep disorder and a potential problem for anesthesia care.[82] This finding is consistent with evidence that obstructive sleep apnea, similar to metabolic syndrome, is a systemic disease.[83] The present results encourage future studies using whole body plethysmography[53] to characterize the actions of intravenous and volatile anesthetics on the respiratory control of LCR and HCR rats. In view of the fact that adenosine is antinociceptive[84,85] and exercise increases brain adenosine,[86] future studies also will determine the extent to which adenosinergic neurotransmission[87] is one mechanism through which intrinsic aerobic capacity modulates sleep and nociception.

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