SHORT NOTE

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Daily Acclimation Handling Does Not Affect Hippocampal Long-Term Potentiation or Cause Chronic Sleep Deprivation in Mice

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Study Objectives: Gentle handling is commonly used to perform brief sleep deprivation in rodents. It was recently reported that daily acclimation handling, which is often used before behavioral assays, causes alterations in sleep, stress, and levels of N-methyl-D-aspartate receptor subunits prior to the actual period of sleep deprivation. It was therefore suggested that acclimation handling could mediate some of the observed effects of subsequent sleep deprivation. Here, we examine whether acclimation handling, performed as in our sleep deprivation studies, alters sleep/wake behavior, stress, or forms of hippocampal synaptic plasticity that are impaired by sleep deprivation.

Design: Adult C57BL/6J mice were either handled daily for 6 days or were left undisturbed in their home cages. On the day after the 6th day of handling, long-term potentiation (LTP) was induced in hippocampal slices with spaced four-train stimulation, which we previously demonstrated to be impaired by brief sleep deprivation. Basal synaptic properties were also assessed. In three other sets of animals, activity monitoring, polysomnography, and stress hormone measurements were performed during the 6 days of handling.

Results: Daily gentle handling alone does not alter LTP, rest/activity patterns, or sleep/wake architecture. Handling initially induces a minimal stress response, but by the 6th day, stress hormone levels are unaltered by handling.

Conclusion: It is possible to handle mice daily to accustom them to the researcher without causing alterations in sleep, stress, or synaptic plasticity in the hippocampus. Therefore, effects of acclimation handling cannot explain the impairments in signaling mechanisms, synaptic plasticity, and memory that result from brief sleep deprivation.

Keywords: Gentle handling, hippocampus, mouse, sleep deprivation, synaptic plasticity

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INTRODUCTION

An important approach to study sleep function is to perform sleep deprivation (SDep) in rodents. This can be carried out using a variety of techniques, which vary in efficacy, specificity for depriving animals of particular sleep stages, and ability to be performed over long durations. In studies aimed to deprive animals as completely as possible over a relatively short period of time (≤ 6 h), gentle handling is a commonly used approach. In this method of SDep, animals are kept awake manually by tapping on the cage, shaking the cage gently, disturbing the nesting material, or by gentle stroking of the animal itself, although this last option is done sparingly.[1-3]

Because this method has the potential to be stressful to the animal, it is often preceded by a number of days of brief handling to acclimate the animal to what it will experience during the SDep period. It is generally acknowledged that handling of mice prior to behavioral procedures is important to habituate animals to handling by the experimenter. For example, in learning paradigms, this acclimation improves learning and limits the association made by the animal between experimenter and the behavioral training itself.[4-7]

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A concern that has been raised recently is that acclimation handling could itself result in behavioral and neurochemical alterations that would affect the subsequent response to SDep.[8] To determine whether the method of acclimation handling used in our studies causes disturbances in sleep or hippocampal function, we have examined sleep architecture, activity patterns, stress, and synaptic plasticity in handled or undisturbed animals. We find that 6 days of gentle handling procedure does not affect forms of long-term potentiation (LTP) that are impaired by brief sleep deprivation. In addition, we show that 6 days of gentle handling does not affect sleep/wake patterns, and that animals habituate to the stress caused by gentle handling. These results indicate that sleep deprivation, rather than prior acclimation handling, is the crucial manipulation producing sleep loss that impairs synaptic plasticity.

METHODS

Animals and Surgery

Adult (2-3 mo old) male C57BL/6J mice were used for all experiments. Mice were individually housed with *ad libitum* food and water on a 12 h-12 h light-dark schedule. All animal care and experiments were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania and conducted in accordance with the National Institutes of Health guidelines. For synaptic plasticity experiments, mice were housed in plastic cages with wire tops. For activity monitoring and polysomnography experiments, mice were housed in noise-attenuating chambers (22″ × 15″ × 14″ for activity monitoring, or 3′ × 4′ × 4′ for polysomnography, Med Associates,

Georgia, VT) with individual lights and fans. Polysomnography was conducted as previously described.[9-11] Animals were implanted with electroencephalography (EEG) and electromyography (EMG) electrodes under isoflurane anesthesia. Electrodes were held in place with dental cement (Ketac, 3M, St Paul, MN). Electrodes consisted of Teflon-coated wires (Cooner wires, Chatsworth, CA) soldered to gold socket contacts (Plastics One, Roanoke, VA) and pushed into a six-pin plastic plug (363 plug, Plastics One). The contacts were cemented to the plug using dental cement. Animals were connected to amplifiers using lightweight cables (363, Plastics One) attached to a rotating commutator (SLC6, Plastics One). All recordings were obtained using either frontal (medial-lateral ML ± 1.5 mm, anterior-posterior AP -2 mm from bregma) or parietal electrodes (ML ± 1.5 mm, AP +1 mm from lambda) referenced to an electrode over the cerebellum (1.5 mm posterior of lambda). Mice were allowed to recover from surgery for a minimum of 2 wk.

Handling

To assess the effects of acclimation handling on synaptic plasticity, activity, sleep-wake patterns, or corticosterone (CORT) levels, one cohort of mice in each experiment was handled daily beginning on the 2nd day of individual housing, whereas the other mice were left undisturbed in their home cages. Acclimation handling was performed at approximately *zeitgeber* time (ZT) 4.5, and lasted 2-3 min per mouse. Acclimation of all animals was completed within 30 min for all experiments. Handling consisted of all techniques used during our gentle handling sleep deprivation method.[1,3] Cages were removed in pairs from their racks and placed on carts for the handling, which included gentle tapping of the exterior of the cage, removing the cage lid, light rattling of the wire cage top and rummaging through the food, removing the wire cage top and gently stroking the mouse as it moved freely around the cage, and disturbing the bedding. In the polysomnography experiment, on the day following the 6th day of handling, animals were sleep deprived for 6 h starting at ZT 0. Animals were sleep deprived using handling as previously described.[1,3] Animals were kept awake by gentle tapping or rattling of the cage, or by removing the wire cage top. Animals were also nudged and their bedding was disturbed, but only in cases when mice did not respond to tapping on the cage.

Electrophysiology

Mice were brought to the electrophysiology room on the day after the last handling session at ZT 5-6 and killed by cervical dislocation, and hippocampal slices were prepared. Electrophysiologic studies were carried out as previously described.[3] Input-output curves were generated by measuring presynaptic fiber volley amplitudes and initial slope of field excitatory postsynaptic potentials (fEPSPs) in area CA1 in response to incremental increases in stimulation intensity up to 30 V in the Schaffer collateral pathway. The slope of the relationship between these variables was calculated. The stimulus strength was then set to elicit 40% of the maximum fEPSP, and paired-pulse facilitation (PPF) was induced by paired stimuli, with interstimulus intervals of 300, 200, 100, 50, and 25 ms. The ratio of the slope of the second response relative to the first was recorded. LTP was induced using a spaced four-train stimulation protocol, consisting of four 1-sec 100-Hz trains with a 5-min interstimulus interval.

Polysomnography

EEG/EMG signals were sampled at 256 Hz and filtered at 0.5-30 Hz and 1-100 Hz, respectively, with 12A5 amplifiers (Astro-Med, West Warwick, RI). Data acquisition and visual scoring was performed using SleepSign software (Kissei Comtec, Inc, Japan). EEG/EMG recordings were scored in 4-sec epochs as wake, non-rapid eye movement (NREM) sleep, or rapid eye movement (REM) sleep by a trained experimenter blind to experimental conditions. Epochs containing movement artifacts were included in the state totals and architecture analysis, but excluded from subsequent spectral analysis. Spectral analysis was performed using a fast Fourier transform (FFT; 0.5-20Hz, 0.125 Hz resolution). NREM slow wave activity (SWA) was computed in 1-h bins and SWA was normalized to the last 4 h of the light phase for each animal as previously described.[12]

Activity Monitoring

Activity was monitored using an infrared beam-break based system (Opto M3, Columbus Instruments, Columbus, OH), which provided a high-resolution grid covering the horizontal plane. The beams were spaced 0.5 inches apart and counts of beam breaks were compiled every 10 sec. In our analysis methodology, if mice did not break a single beam in the horizontal plane for 40 sec or longer, the animals were considered asleep. We chose this threshold because it was validated in a previous study using the same activity monitoring system.[13] We also reanalyzed the data, setting the inactivity threshold to 60, 90, 120, or 180 sec, but did not observe any difference in the effects of handling on activity for these various threshold values.

Blood CORT Measurements

On day 1 and day 6 of handling, trunk blood was collected 20-30 min after handling in pre-cooled plastic centrifuge tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Blood was centrifuged for 15 min at 2,600 rpm and plasma was stored at -80ºC until further processing. CORT levels were determined by radioimmunoassay, according to kit instructions (MP Biomedicals, Orangeburg, NY).

Statistics

All electrophysiology data were analyzed with SigmaStat (Systat Software, San Jose, CA). For input-output and maximum response data, one-way analyses of variance (ANOVAs) were used, with handling as the main factor. For PPF data, a repeated-measure ANOVA was used, with interval as the within-subject factor and handling as the between-subject factor. For LTP analysis, a repeated-measure ANOVA compared fEPSP slopes during the first 20 min after the last tetanus (induction), and during the last 20 min of the recordings (maintenance), normalized against baseline, with time as the within-subject factor and handling as the between-subject factor. SAS software version 9.2 (SAS Institute Inc., Cary, NC) was used to analyze all activity, blood CORT levels, and polysomnography data. For activity monitoring, the estimated time spent asleep was analyzed using Student's *t*-tests to compare handled versus non-handled groups. For polysomnography experiments, one-way

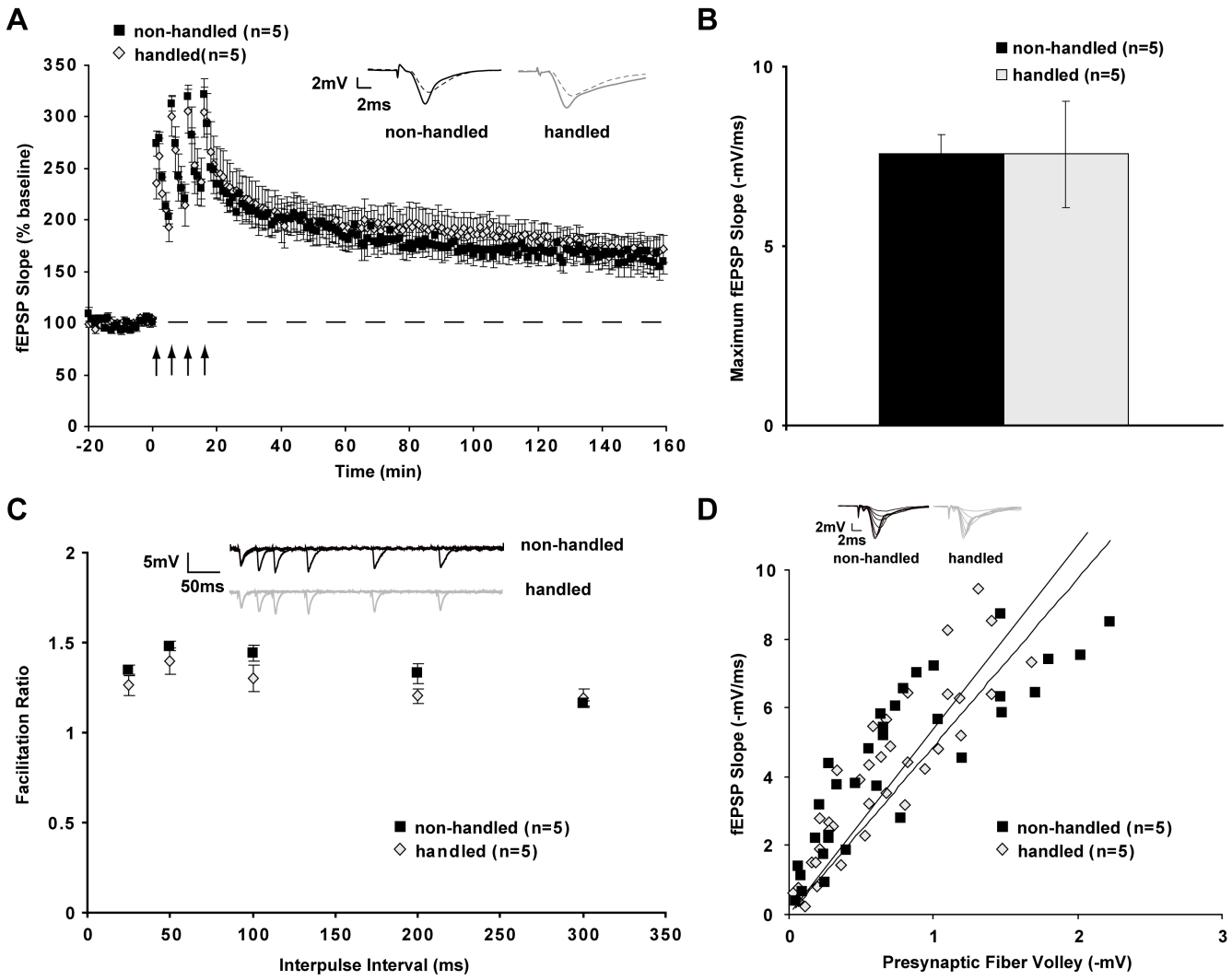

Figure 1—Handling does not disrupt hippocampal plasticity. Handling did not affect induction or maintenance of spaced four-train long-term potentiation **(A)**, as measured by field excitatory postsynaptic potentials (fEPSPs) from hippocampal Schaffer collateral CA1 synapses. Inset shows representative traces depicting fEPSP responses during baseline (dashed lines) and at 160 min posttetanization (solid lines). Handling also did not alter basal synaptic properties, such as the maximum response amplitude **(B),** paired-pulse facilitation (PPF) **(C)**, or input-output relationship **(D)**. Insets in C and D show overlaid representative fEPSP responses from PPF and I/O experiments, respectively. Shown are means ± standard error of the mean.

ANOVAs were used to compare time in wake, NREM sleep, and REM sleep following handling. Repeated-measure ANOVAs were used to analyze sleep rebound and SWA with time or day as a within-subject factor and treatment as a between-subject factor. Tukey's range tests were used to compare SWA at each time point during the 6th day of handling and following SDep. Data collected during the light phase and dark phase were analyzed separately for all analyses. For blood CORT levels, we used a two-way ANOVA with "treatment" and "day" as between-subject factors, followed by Tukey *post hoc* tests.

RESULTS

To determine the effect of acclimation handling on hippocampal synaptic plasticity, we first compared the effect of handling on spaced four-train LTP, a protein kinase A-dependent, postsynaptically expressed form of plasticity that is impaired by brief sleep deprivation.[3] We found that acclimation handling did not affect the induction (no overall effect of handling, $F = 0.13$, $P = 0.73$) or maintenance (no overall effect of handling, $F = 0.07$, $P = 0.8$) of LTP (Figure 1A). We also assessed the effects of handling on basal synaptic properties, including the maximum response amplitude (Figure 1B), paired-pulse facilitation (Figure 1C), and input-output relationship (Figure 1D). No effects of handling were observed on any of these measures (Max response—no overall effect of handling, $F = 0.17$, $P = 0.69$; PPF—no overall effect of handling, $F = 5.16$, $P = 0.053$, and no interaction between handling and interstimulus interval, $F = 1.31$, $P = 0.29$; Input-Output—no effect of handling, $F = 0.25$, $P = 0.63$). There was a nonsignificant trend toward an overall reduction in PPF in handled animals, which could be representative of a minor alteration in presynaptic transmitter release dynamics.[14] However, we previously found that PPF was unchanged by sleep deprivation in handled animals,[3] and have shown here that a form of LTP that is impaired by sleep deprivation is completely unaffected by handling. Therefore, we do not believe that this trend represents a relevant physiologic change that could explain effects of sleep deprivation on LTP. These findings

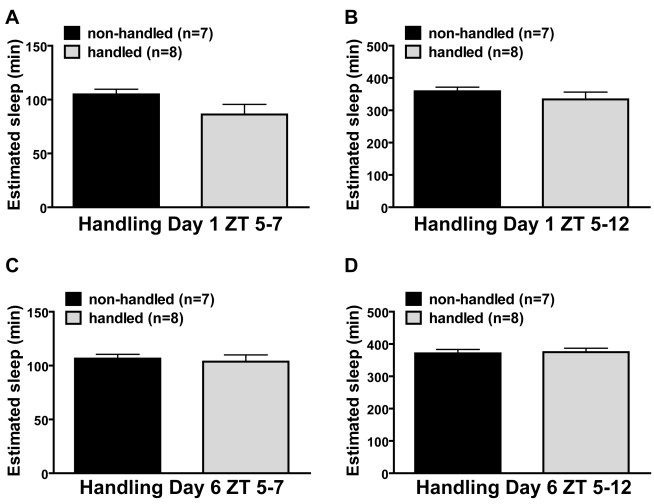

Figure 2—Handling does not alter activity patterns. One the 1st day of handling, estimated sleep was not altered by handling for the first 2 h posthandling **(A)** or during the remainder of the light phase **(B)**. Estimated sleep was similar between the two groups on the 6th day of handling in the first 2 h posthandling **(C)** or during the remainder of the light phase **(D)**. Shown are means ± standard error of the mean.

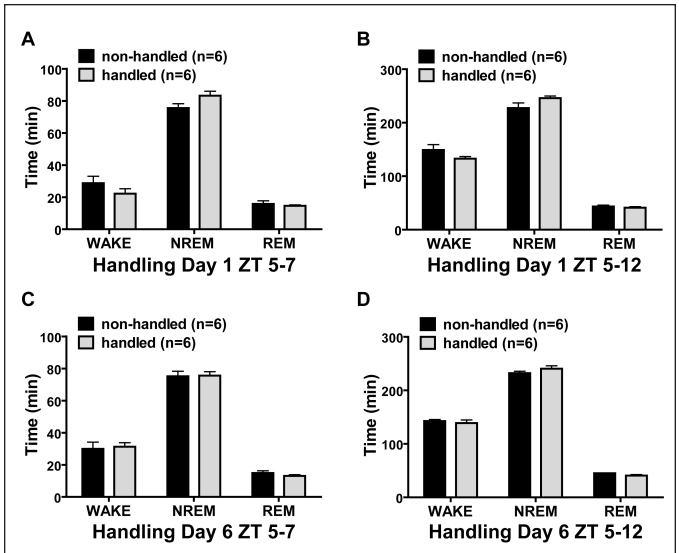

Figure 3—Time spent awake, in non-rapid eye movement (NREM) sleep, or in rapid eye movement (REM) sleep are not changed following handling. Time spent in wake, NREM sleep, and REM sleep was not altered in the 2 h **(A)** or 7 h **(B)** following the 1st day of handling. Wake, NREM sleep, and REM sleep were also unaffected in the 2 h **(C)** and 7 h **(D)** following the 6th day of handling. Shown are means ± standard error of the mean.

demonstrate that brief acclimation handling alone for 6 days does not produce any effects on synaptic plasticity or baseline synaptic function that could explain the deficits observed due to sleep deprivation.

Next, we determined the effect of daily acclimation handling on rest/activity patterns assessed by a validated activity monitoring system.[13] On the first day of handling, estimated sleep time was unchanged, during the first 2 h immediately following brief acclimation handling (Figure 2A, P = 0.1127) and during the remainder of the light phase (Figure 2B, P = 0.3717). On the 6th day of handling, estimated sleep levels were also similar between handled versus control mice, during the 2 h immediately following handling (Figure 2C, P = 0.7158) and during the remainder of the light phase (Figure 2D, P = 0.8382). Handling had no effect on the rest-activity pattern during the subsequent dark phase on day 1 (P = 0.2) or day 6 (P = 0.54) (data not shown). These analyses were repeated using different thresholds for sleep, as described in the Methods section, and all versions showed a complete noneffect of acclimation handling on rest time during the following 7 h of the light phase and during the dark phase. These findings suggest that handled animals are not chronically sleep deprived and that daily handling does not significantly alter rest/activity patterns.

We also used polysomnography to examine whether handling alters particular stages of sleep or sleep/wake architecture. Time spent awake was unchanged in the first 2 h following the first day of handling ($F(1,10) = 1.54$, $P = 0.2423$, Figure 3A). NREM sleep ($F(1,10) = 3.92$, $P = 0.0760$) and REM sleep ($F(1,10) = 0.33$, $P = 0.5797$) were also unchanged during the first 2 h following the 1st day of handling. Wakefulness ($F(1,10) = 2.32$, $P = 0.1585$), NREM sleep ($F(1,10) = 3.16$, $P = 0.1059$) and REM sleep ($F(1,10) = 0.54$, $P = 0.4792$) were also unaltered during the remainder of the light period following the 1st day of handling (Figure 3B). On the 6th day of handling, wakefulness ($F(1,10) = 0.08$, $P = 0.7888$), NREM sleep ($F(1,10) = 0.01$, $P = 0.9180$), and REM sleep ($F(1,10) = 0.33$, $P = 0.5797$) were unchanged during the first 2 h after handling (Figure 3C) and during the remainder of the light period (Figure 3D; wake: $F(1,10) = 0.32$, $P = 0.5851$; NREM sleep: $F(1,10) = 1.64$, $P = 0.2287$; REM sleep: $F(1,10) = 0.35$, $P = 0.5863$). Handling did not change the number of episodes of wake, NREM sleep, or REM sleep, and did not alter the episode duration for any of these behavioral states (Table 1). These results indicate that acclimation handling had no significant effect on sleep/wake architecture on the 1st day or on the 6th day of handling (Table 1). On the 7th recording day, animals were sleep deprived for 6 h, starting at lights on (ZT 0), to examine whether gentle handling would affect sleep homeostasis. NREM sleep rebound was equivalent for handled and non-handled animals (data not shown, handling: $F(1,9) = 0.37$, $P = 0.5598$, time × handling $F(5,45) = 1.67$, $P = 0.1825$). REM sleep rebound was also similar between the two groups (data not shown, handling: $F(1,9) = 0.04$, $P = 0.8460$; handling × time: $F(5,45) = 0.82$, $P = 0.5418$).

SWA, the spectral power of the EEG in the 0.5-4 Hz range during NREM sleep, is the best-characterized marker of sleep intensity and changes in response to sleep loss.[15,16] A repeated-measure ANOVA revealed that SWA decreased over the course of the light phase for both handled and undisturbed animals on the 6th day of handling ($F(11,110) = 33.32$, $P < 0.0001$, Figure 4A), and that SWA was not different between handled and undisturbed mice (time × treatment $F(11,110) = 1.70$, $P = 0.1735$). Handled animals showed a small but significant (Tukey range test, $P < 0.05$) increase in SWA in the hour following handling when tested on the 6th day (Figure 4A). Handled animals showed identical SWA to undisturbed mice by the next hour (Figure 4A), suggesting that handled animals recover within 1 h after handling. During recovery following SDep, there

Table 1—Handling does not alter sleep/wake architecture

	Handling day 1			Handling day 6		
WAKE	**Non-handled**	**Handled**	**P**	**Non-handled**	**Handled**	**P**
Number of bouts	128.17 ± 13.94	134.83 ± 6.63	0.6749	143.33 ± 16.02	156.33 ± 12.46	0.5362
Avg duration (sec)	252.33 ± 36.97	173.83 ± 10.36	0.0712	214.67 ± 22.94	161.5 ± 23.54	0.1369
NREM	**Non-handled**	**Handled**	**P**	**Non-handled**	**Handled**	**P**
Number of bouts	194.83 ± 27.84	213.83 ± 10.57	0.5378	222.67 ± 25.52	248.5 ± 21.23	0.4545
Avg duration (sec)	61.67 ± 8.10	57.5 ± 4.33	0.6598	50.00 ± 4.99	51.67 ± 6.40	0.8415
REM	**Non-handled**	**Handled**	**P**	**Non-handled**	**Handled**	**P**
Number of bouts	49.17 ± 7.81	43.00 ± 3.16	0.4811	44.67 ± 2.39	41.67 ± 2.12	0.3702
Avg duration (sec)	43.83 ± 2.56	45.17 ± 2.00	0.6906	46.33 ± 1.67	48.5 ± 2.70	0.5108

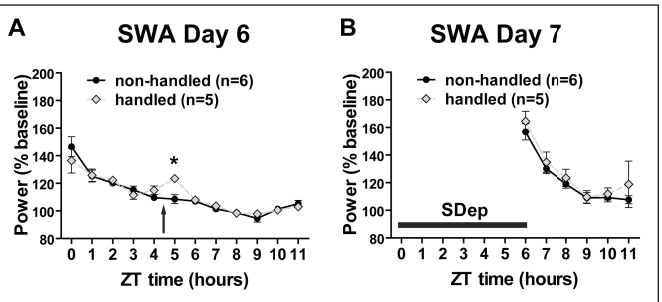

Figure 4—Sleep homeostasis is unaffected by handling. Slow wave activity (SWA, the power from 0.5-4 Hz during non-rapid eye movement sleep) was computed during the light phase on the 6th day of handling **(A)**. The arrow indicates when handling occurred on day 6. SWA increased in the hour following handling on Day 6. SWA was also calculated following 6 h of sleep deprivation on the 7th day of recording **(B)**. The dark bar indicates when animals were sleep deprived (SDep). Shown are means ± standard error of the mean. The asterisk represents $P < 0.05$.

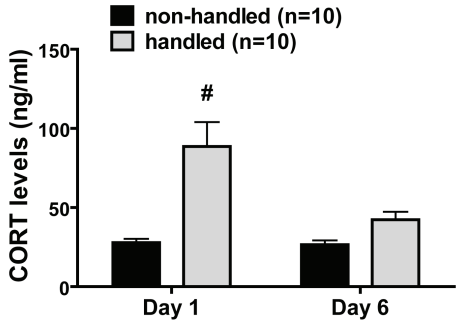

Figure 5—Animals habituate to stress related to handling. Blood corticosterone (CORT) levels were measured after 1 day and after 6 days of handling, compared with non-handled control animals (n = 10 per group). Blood CORT levels were higher for handled animals compared to control animals on the first day of handling but not on the 6th day of handling. Shown are means ± standard error of the mean. The number sign represents $P < 0.0001$.

was no difference in SWA between handled and non-handled animals (Figure 4B, treatment: $F(1,9) = 0.60$, $P = 0.4584$; time × treatment: $F(5,45) = 0.31$, $P = 0.7554$) and both groups of mice showed increased SWA, which declined over time (time: $F(5,45) = 34.47$, $P < 0.0001$).

To determine the effects of acclimation handling on the stress response in mice, we measured blood CORT levels 20-30 min after the 1st and 6th days of handling. The effect of handling on CORT levels was reduced on Day 6 compared with Day 1 (treatment × day $F(1,36) = 7.26$, $P = 0.0107$) (Figure 5). *Post hoc* tests showed that CORT levels were significantly higher in handled mice compared with those of control mice on the 1st day of handling ($P < 0.0001$). In contrast, CORT levels in handled mice were similar compared to those of control mice on the 6th day of handling ($P = 0.5474$), suggesting that animals acclimated to the stress induced by this manipulation.

DISCUSSION

Because acclimation handling is often used in the days leading up to SDep and many behavioral experiments, it is important to know if the acclimation handling causes chronic changes that could contribute to the behavioral and physiologic responses to SDep. To address this issue, we determined whether brief acclimation handling for multiple days affects hippocampal synaptic plasticity or alters sleep-wake states in C57BL/6J mice. We found that a protein kinase A-dependent form of LTP previously shown to be affected by 5 h of sleep deprivation was not affected by multiple days of handling. In addition, we show that brief acclimation handling does not cause a prolonged disruption of sleep, change the homeostatic response of mice to subsequent sleep deprivation, or produce chronic alterations in stress.

In previous studies aimed at determining the effects of sleep deprivation on memory and synaptic plasticity, animals were handled for multiple days prior to experimentation.[1,3] These studies found significant differences between acclimation-handled, non-sleep-deprived animals and acclimation-handled, sleep-deprived animals, suggesting that the molecular changes and memory deficits occurred as a result of the sleep deprivation, not the handling. In addition, we found that rescuing cyclic adenosine monophosphate (cAMP) signaling, by treatment with rolipram during the sleep deprivation period, prevented memory deficits in sleep-deprived animals.[3] It is highly unlikely that molecular events occurring during previous days of brief acclimation handling were responsible for the memory deficits observed following sleep deprivation. In line with the idea that gentle handling alone is not a major contributor to the observed effects of SDep on memory, a recent paper by Hagewoud et al.[2] showed that the negative effect of SDep on contextual fear conditioning was independent of the amount of stimulation (e.g., handling) the animals received. This finding indicates that the memory deficits observed in their study were not due to gentle handling, but rather due to SDep.

Conversely, a recent study by Longordo et al.[8] reported a surprisingly large (25%) decrease in resting time, measured by activity monitoring, during the 7 h following daily acclimation handling. They also found increased stress hormone levels by the end of 6 days of daily handling. The authors argued that repeated daily handling was the equivalent of chronic partial sleep deprivation as well as chronic stress, which could clearly be a detriment for many forms of subsequent behavioral testing. Thus, in the current report, we attempted to confirm or deny these conclusions using acclimation handling as carried out in our previous studies.

In contrast to the report by Longordo et al.,[8] we did not find any effect of handling on sleep time across the same 7-h period following handling. Even when we used activity monitoring to estimate sleep time during the first 2 h after acclimation handling, when one might expect handling to have the largest effect on sleep, there was no significant effect. Because activity monitoring can be an imperfect measure of sleep, we went on to use polysomnography, which showed that handling did not alter NREM or REM sleep, or change sleep/wake architecture. Chronic sleep deprivation has been shown to enhance homeostatic sleep rebound and slow wave activity (SWA).[17] We did not observe any difference in sleep rebound or SWA following 6 h of SDep in handled animals compared with control animals, consistent with our findings that handling does not disrupt sleep/wake levels or sleep architecture. We also measured blood CORT levels 20-30 min after handling, at a point when stress hormone levels are known to peak following a stressor.[18] Animals habituated to handling by the 6th day, and the blood CORT levels were overall very low and did not exceed normal circadian peak values.[19] These results indicate that daily handling is not equivalent to chronic sleep loss or stress.

Although unlikely, it is possible that a complex interaction between handling and sleep deprivation is required for sleep deprivation to cause the effects that we have observed in our recently published work,[3] including disrupted cAMP signaling, impaired synaptic plasticity, and hippocampus-dependent memory. However, another independent group of researchers has observed that brief sleep deprivation causes specific deficits in hippocampal memory, as well as impairments in the extracellular signal-regulated kinase signaling pathway, which is tightly linked to cAMP signaling. Importantly, their work was done without any acclimation handling prior to the sleep deprivation procedure,[20] suggesting that the crucial manipulation is sleep deprivation, not prehandling.

These data indicate that it is possible to perform acclimation handling that does not cause unwanted changes in sleep behavior or hippocampal function. Indeed, a recent study suggests that seemingly small differences in handling technique can cause largely varying responses in stress signaling and anxiety.[21] The handling methods used in our studies and the study by Longordo et al.[8] may differ enough to account for the difference in observed effects on sleep, but as yet it is unclear what aspect of their handling methodology could have resulted in altered sleep and chronic stress.

Longordo et al.[8] state that they want to "contribute to the standardization of SDep procedures." However, for laboratories interested in studying animal behaviors such as learning and memory, or using injections or any other procedure that requires handling, acclimation handling is crucial. Indeed, handling is widely conducted prior to behavioral analysis and has been shown to reduce anxiety.[6] This is corroborated by our measurements of CORT levels after handling, which show that mice grow accustomed to handling over the course of 6 days of handling (Figure 5). Handling also ensures that behavioral responses are task-specific, rather than associated with the exposure to experimenters or novel environments.[7] Finally, lack of handling can negatively affect performance in memory tasks.[4,5] Therefore, the goal for studies that rely on acclimation handling should be to include comparisons in their studies to ensure that such handling does not alter baseline behavior or neural plasticity.

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DISCLOSURE STATEMENT

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