Progesterone Sharpens Temporal Response Profiles of Sensory Cortical Neurons in Animals Exposed to Traumatic Brain Injury

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Abstract

Traumatic brain injury (TBI) initiates a cascade of pathophysiological changes that are both complex and difficult to treat. Progesterone (P4) is a neuroprotective treatment option that has shown excellent preclinical benefits in the treatment of TBI, but these benefits have not translated well in the clinic. We have previously shown that P4 exacerbates the already hypoactive upper cortical responses in the short-term post-TBI and does not reduce upper cortical hyperactivity in the long term, and we concluded that there is no tangible benefit to sensory cortex firing strength. Here we examined the effects of P4 treatment on temporal coding resolution in the rodent sensory cortex in both the short term (4 d) and long term (8 wk) following impact-acceleration–induced TBI. We show that in the short-term postinjury, TBI has no effect on sensory cortex temporal resolution and that P4 also sharpens the response profile in all cortical layers in the uninjured brain and all layers other than layer 2 (L2) in the injured brain. In the long term, TBI broadens the response profile in all cortical layers despite firing rate hyperactivity being localized to upper cortical layers and P4 sharpens the response profile in TBI animals in all layers other than L2 and has no long-term effect in the sham brain. These results indicate that P4 has long-term effects on sensory coding that may translate to beneficial perceptual outcomes. The effects seen here, combined with previous beneficial preclinical data, emphasize that P4 is still a potential treatment option in ameliorating TBI-induced disorders.

Introduction

The pathophysiological consequences of traumatic brain injury (TBI) are complex and difficult to treat,[1] and despite research into therapeutic options, mortality rates following severe TBI are still high.[2] Progesterone (P4) is one treatment candidate that has shown excellent preclinical potential and some benefits for the treatment of mild TBI in humans.[3,4] In animal studies, it has been shown to influence many of the complex pathological changes that occur in TBI. In the present study, we focused only on the changes in diffuse TBI, which was the specific model studied here. Diffuse TBI results from acceleration/deceleration of brain tissue causing immediate axonal injury and subsequent atrophy—injuries that are undetectable via traditional imaging techniques.[5–10] Initial axotomy is followed by cytoskeletal damage, impairment of axonal transport, and an increase in axon diameter that contribute to secondary axotomy in the hours immediately after impact. Further short-term pathological cascades

lead to dysfunction of the axolemma, mitochondrial swelling, disruption to axon diameter, loss of microtubules, myelin loss and breakdown, and finally axonal disconnection.[11] There is also a wave of spreading depression[12,13] and evidence of oxidative stress in the early postinjury stages, which may cause later synaptic malfunction and alter plasticity.[14] TBI-induced pathologies continue to evolve over months and include the

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development of new, inappropriate synaptic connections and inhibitory cell loss which are thought to contribute to functional morbidities.[15,16] P4 produces benefits from these pathologies through effects including reduced oedema,[17] improved mitochondrial function,[18] reduced inflammation,[19] reduced necrotic damage,[20] reduced excitotoxicity,[21] and protection of the blood–brain barrier (BBB).[22]

In contrast to these encouraging results, preclinically and in human mild TBI, P4 has shown only equivocal results in the treatment of moderate to severe TBI in phase III clinical trials.[23] This was also the case in our previous study on the effects of P4 on TBI-induced changes in neuronal function in the sensory cortex.[24] We have conducted a series of electrophysiological studies on the effects of diffuse TBI, generated using an impact-acceleration injury model,[25] on neuronal encoding in the rodent barrel cortex.[24,26,27] This cortical area processes input from the large facial whiskers that provide the tactile input used to navigate and discriminate the world and to interact with conspecifics. We found that immediately (24 h)[26] and soon after (4 d)[24] diffuse TBI, neural responsiveness in the supragranular cortical layers was suppressed, but this changed in long-term TBI[27] to a relative hyperexcitation in the same layers. Against this baseline, we tested short- and long-term effects of P4 treatment in the same model of diffuse TBI[24] and found that, contrary to our expectations of benefits, P4 exacerbated the effects of diffuse TBI and reduced the already hypoactive firing rates in upper cortical layers at 4 d postinjury and in the long term had no benefits for the hyperexcitable neural responsiveness.

In our previous cortical studies, we indexed neuronal functionality in the response rate, studying effects on the neuronal response (firing) rate to simple and complex stimuli varying in critical parameters of whisker motion (whisker motion velocity or amplitude). Neuronal encoding is defined not only by response strength but also by the temporal patterns of neuronal firing. In light of the suggestion that the absence of P4 effects in human moderate and severe TBI is likely because effective dosing regimens and outcome metrics have yet to be properly defined,[1,28] in the present study, we reexamined that database[24] with new metrics of the temporal features of neuronal responses. We now report that P4 sharpens the temporal features of cortical response profiles in the short- and in the long-term postinjury. These novel results suggest that P4 treatment may produce benefits for sensory encoding features dependent on temporal patterns of neuronal responses—such as stimulus discriminability—in diffuse TBI. Furthermore, they suggest that outcome measures and performance metrics based on temporal features of behavior may reveal P4-induced benefits in the treatment of TBI.

Materials and Methods

Animals

Eight- to 10-wk-old male Sprague-Dawley (SD) rats (Monash Animal Research Platform, Melbourne, Australia) weighing between 330 and 350 g were housed with ad libitum access to food and water under a 12-h light–dark cycle. After 1 wk of acclimatization, animals underwent either diffuse TBI or sham control surgery. All experimental procedures adhered to the National Health and Medical Research Council of Australia guidelines and were approved by the Monash University Animal Ethics committee.

TBI/Sham Surgery

This study reexamines, with new analyses, data from animals we reported on previously regarding the effects of P4 on TBI.[24] As such, surgical and electrophysiological techniques will only be touched on briefly. A total of 24 animals were treated with the weight-drop impact-acceleration method[25] modified as described,[29] and 27 animals underwent sham surgery. All animals were anesthetized in a closed system box with 5% isoflurane via inhalation; when deeply anesthetized and impervious to strong noxious pinching, they were intubated and ventilated with a maintenance dose of 3.5% isoflurane in 22% oxygen/78% nitrogen. A metal disc (1 cm diameter, 3 mm thick) was fixed on the exposed skull between bregma and lambda. The animal was placed on a foam bed and a 450-g weight was dropped 2 m through a vertical tube onto the metal disc. The specific technique for applying TBI we used here was described and developed elsewhere, and we used an identical model with impact velocity calibrated to 6.15 m/s to induce severe injury.[30] Following impact, ventilation was resumed with 22% oxygen/78% nitrogen until the animal was capable of self-respiration. The scalp incision was sutured and sterilized, and animals were left to recover. Sham surgery was identical but did not involve the weight-drop procedure. The temperature of the animal was maintained at 37 to 38 °C during all surgical procedures.

P4 Treatment Regime

Levels of endogenous P4 fluctuate around the ovarian cycle in female rats.[31] Thus, we only used male rats in this study. Animals were randomly assigned to receive either P4 in peanut oil (Sigma-Aldrich, St. Louis, MO; 16 mg/kg; ~ 0.6 mL) or vehicle treatment (peanut oil only, ~ 0.6 mL; Sigma-Aldrich) postsurgery. The dosing regimen was based on previous studies, indicating that 16 mg/kg provided cognitive benefits and a reduction in apoptotic markers to brain-injured animals[19,32–35] and was consistent with a clinical dosing regimen that had some success.[4] The first drug or vehicle injection (as appropriate) was given intraperitoneally within the first hour postsurgery, followed by subcutaneous injections at 6 and 24 h. In the short-term survival group (details below), no further injections were given and animals were left to recover for 4 d postsurgery until the electrophysiological study. In the long-term group, subcutaneous injections were given 1 wk postsurgery and then weekly until electrophysiological recordings occurred at 8 wk postsurgery.

There were 4 sham groups, namely, the sham + peanut oil–treated groups (Sham + Veh, $n = 7$ animals for 4 d postsurgery survival, $n = 7$ for 8 wk postsurgery survival) and sham + P4 groups (Sham + P4, $n = 8$ for 4 d post-TBI survival, $n = 5$ at 8 wk post-TBI survival), and 4 TBI groups, namely, TBI + peanut oil–treated groups (TBI + Veh, $n = 7$ at 4 d post-TBI survival, $n = 6$ at 8 wk post-TBI survival) and the TBI + P4 groups (TBI + P4, $n = 6$ at 4 d post-TBI survival, $n = 7$ at 8 wk post-TBI survival).

Surgery to Record from Barrel Cortex

Electrophysiological recordings were obtained from the barrel cortex at 4 d or 8 wk post-TBI or sham surgery, using identical surgical procedures for data acquisition. Animals were placed in a closed system box and anesthetized via 5% halothane (Sigma-Aldrich, St Louise, MO, USA) in oxygen. When they had attained deep anesthesia (i.e., absent withdrawal reflexes to strong forepaw pinch or palpebral reflexes), they were tracheotomized for continuous ventilation with 0.5% to 3.0% halothane (Sigma-Aldrich) in oxygen. Anesthetic depth was monitored via continuous electrocardiogram/electromyogram recordings and regular monitoring of pinch withdrawal and palpebral reflexes. A thermostatically controlled heating blanket with feedback control from a rectal probe maintained body temperature at 37 to 38 °C (Model TR-100, Fine Science Tools, Foster City, CA).

A midline cranial incision was used to expose the skull surface widely and a head bar secured firmly into the skull rostral of bregma using a stainless steel screw and dental acrylic. A craniectomy exposed the right barrel cortex (~ 2 mm caudal to bregma, 6 mm lateral to the midline) with dura left intact. Under high-power microscopy, a tungsten microelectrode (2 to 4 MΩ; FHC, Bowdoin, ME), held in a calibrated microdrive (Model 2660, David Kopf Instruments, Tujunga, CA, USA) mounted on a custom rig of translators and goniometers,[36,37] was positioned to make light contact with the pia. The microdrive was zeroed with the microelectrode tip placed on the cortical surface, and the microdrive was used to rapidly advance the electrode to between 600 and 800 μm from the surface which has been explained in more detail elsewhere.[37] The electrode was allowed to settle in place here and then the principal whisker (PW; the whisker providing main excitatory input) was determined from manual whisker deflections. If a single PW was identifiable, further recordings were obtained with stimuli applied under automated control; if unidentifiable, the microelectrode was removed from the cortex and repositioned elsewhere to repeat the process until a single PW could be identified in a recording site. As detailed more thoroughly elsewhere,[36,37] for computer-controlled stimulus delivery, the PW was attached to a motorized lever arm (Aurora Scientific Inc., Aurora, Ontario, Canada) which could move the whisker in any desired motion, with optical feedback of movement allowing for precise registration of neural activity to stimulus phase.

Neuronal activity was filtered and amplified (Model 2400, Dagan Corporation, Minneapolis, USA; 1000x gain, bandpass filter 300 Hz to 10 kHz) and then enhanced via a graphic equalizer (Rane Corporation, Mukilteo, WA, USA; bandpass gain: +12 dB from 800 Hz to 6kHz, 0 dB at 630 Hz and 8 kHz, and −15 dB at 25-500 Hz and > 10 kHz) as explained elsewhere,[36,37] monitored on an oscilloscope, and played out via speakers. A Schmitt trigger set voltage levels for spike triggerings for online generation of raster and peristimulus time histograms (PSTHs) during stimulus presentation. Online PSTHs were compared to our laboratory database to ensure the depth-defined responses aligned with postmortem histological verification of laminar location, providing online physiological verification of lamina alongside the microdrive depth recordings which were also recorded. Spike waveforms and triggers were stored on the computer which also generated the whisker motion stimuli, for later off-line analyses.

Electrophysiological Data

At each recording location, a suite of 5 trapezoid stimuli[26] was first applied to characterize neuronal responses. The trapezoid stimuli differed in the onset ramp velocity, at 30, 60, 150, 250, or 400 mm/s, with deflection amplitude always fixed at 3.6 mm, trapezoid hold duration always at 20 ms, and offset ramp duration always at 40 ms; stimuli were presented with a 1-s interstimulus interval. Each trapezoid was presented 100 to 250 times pseudorandomly. Spike sorting was done online using the Spike 2 spike sorting algorithms (CED Spike 2).[36,37]

Then we presented 2 complex, naturalistic whisker deflections to the PW. The deflections were played out from text files which stored stimulus characteristics, using our whisker motion system (see Alwis, Yan, Morganti-Kossmann, and Rajan[27]). The first complex motion, the object contact stimulus, was modeled on whisker motion video recorded from awake exploring rats making contact with a rod placed in the path of the whiskers.[38] The second, the rough surface stimulus, was based on video of the whisker of an awake trained rat brushing across a rough surface.[39] Ten stimulus amplitudes were used for each complex whisker motion, with the lowest amplitude being 0.2 mm, then 0.4 mm, and thereafter increasing from 0.4 mm intervals to 3.6 mm. Each amplitude was presented 50 times in a pseudorandom order, with a 1-s interstimulus interval between successive stimuli.

We aimed to collect data from 10 multiunit neuronal clusters from each animal, from each cortical laminar, with adjacent recordings separated by at least 100 μm in cortical depth; 2 clusters recorded from each cortical layer were defined as follows: layer 2 (L2): 150 to 300 μm from pia; upper layer 3 (U3): 350 to 500 μm; deep layer 3 (D3): 550 to 700 μm; layer 4 (L4): 750 to 1,000 μm; and layer 5 (L5): 1,100 to 1,400 μm. For each cluster, we applied the trapezoid, the object contact stimulus, and the rough surface stimulus suites in succession. Recently, we have shown that there is a change in L4 sensory cortex thickness at 8 wk post-TBI when applying the same weight-drop method used

here.[16] However, despite this change in L4 thickness, our recording depths still fall within L4 and, combined with our microdrive calibrations and our extensive database of electrophysiological recordings, allowed us confidence in collating laminar-specific recordings.

Data Analyses

Neural responses were collected in a window of 5 to 50 ms poststimulus onset to encompass the entire duration of all stimuli. In the new analyses here, we considered the temporal aspects of responses to the stimuli, using a new response dispersion metric (RDM) and a previously defined metric, the half peak width (HPW),[26] as explained below.

For each multiunit cluster, we first calculated the firing rate in 1-ms bins across the 50-ms window, to generate the PSTH of firing rate versus time. These firing rates were then summed across the 50 bins to obtain the excitatory area under the curve (EAUC).[26] A comparison of EAUC could provide information about the total response strength in the 50-ms window but could be confounded by any change in the neuronal peak firing rate (PFR). Hence, we divided each multiunit EAUC by the corresponding PFR to obtain a *normalized EAUC* score for each stimulus for each multiunit cluster (normalized total response strength). We then pooled data for clusters from the same cortical layer and experimental group. We term this normalized EAUC the RDM, the normalized summed firing rate across the entire stimulus window; a small RDM indicates a narrow response profile around the PFR; and a large RDM indicates the relative response profile was broad. The temporal width of the EAUC at the firing rate which was half the maximum value was taken as the HPW. Note that for any given cluster, the HPW is the same whether calculated from the normalized or nonnormalized PSTHs; thus using this metric in conjunction with the RDM, which is scaled to normalize EAUC for PFR, provides detailed information on the temporal response profiles of the cluster. Examples of grand PSTHs and normalized grand PSTHs are shown in Fig. 1 to illustrate the difference in firing strength between TBI and sham animals and the RDM and the HPW. Although the mean firing rate PSTH and the normalized PSTH response profiles in the figure come from the same neural population, the profiles differ as the mean PSTH is influenced by firing rate variation across the clusters in the population, whereas the normalized PSTH is not affected in this way. The RDM combined with the HPW allowed us to examine whether TBI and/or P4 altered firing temporal profiles independent of the PFR changes we reported previously.[24,27]

For trapezoid stimuli, these metrics were calculated separately for each particular onset ramp velocity, and for the object contact whisker and rough surface stimuli (data not shown in figures), metrics were calculated separately for each stimulus amplitude.

For each lamina, statistical comparisons between groups were made using 2-way analysis of variance (ANOVA), with
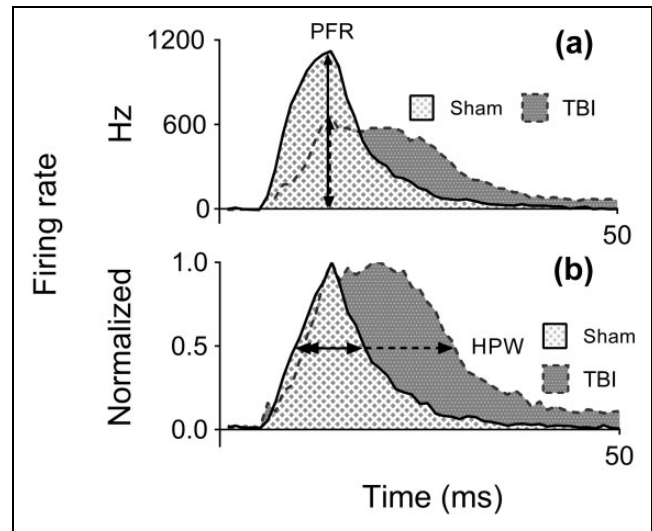
Figure 1. Grand peristimulus time histograms (PSTHs) from Sham + Vehicle (Veh) and traumatic brain injury (TBI) + Veh animals at 8 wk postsurgery in layer 5 (L5) in response to the trapezoid stimulus. (a) The 450-mm/s ramp speed trapezoid-driven Grand PSTHs are presented here to visualize response profiles following the effects of either TBI or sham surgery in the long-term survival group and illustrate the difference in firing strength between sham and TBI animals. (b) Corresponding normalized Grand PSTHs illustrate the difference in the response profile that is not dictated by neuronal peak firing rate (PFR). The firing patterns give a clear indication of the onset response to the ramp element of the stimulus which occurs in the first 20-ms poststimulus onset. The width of these firing patterns provides information regarding the temporal profile of whisker-driven neural activity including the sustained firing times. The area under the curve of PSTHs was divided by the PFR and used to calculate the response dispersion metric (RDM) which provides information about the relative firing rate around the PFR and was used in this research to compare response profiles between experimental groups. Highlighted is the area under grand PSTHs used to represent the mean RDM (b) for sham and for TBI animals in responses recorded from L5. Arrows in (b) indicate region of curve used to calculate half peak width.

experimental group and stimulus velocity (for the trapezoids) or amplitude (for the complex stimuli) as the independent variables and RDM or HPW as the dependent variable. When there was a significant interaction effect, we applied Holm-Šídák post hoc tests, with adjustments for multiple comparisons to identify velocity or amplitude-specific differences. Compared to other common multiple comparison tests such as Bonferroni, Holm-Šídák post hoc adjustments are more precise and powerful.[19] All statistical comparisons were conducted using Matlab (MathWorks, Natick, MA, USA).

Previously, we published a paper using the same animals used in this study to show that P4 attenuated firing strength in the short-term postinjury.[24] These animals were also assessed for motor skills and sensory-based anxiety as a function of TBI, and the results showed significant and persistent sensorimotor deficits following TBI that were not attenuated by P4, but that there was some relief of sensory-based anxiety for TBI animals treated with P4. The

behavioral results are not represented here but can be seen in our previous complementary paper.[24]

Results

Electrophysiological recordings from barrel cortex were collated as being from multiunit clusters in L2 (150 to 300 μm from pia), U3 (350 to 500 μm), D3 (550 to 700 μm), L4 (750 to 1,000 μm), or L5 (1,100 to 1,400 μm). From the responses to the simple trapezoid stimulus and the 2 complex stimuli for each multiunit cluster, we calculated the dispersed response strength metric, the RDM, of the normalized EAUC across the stimulus window, and the temporal dispersion of the responses, the HPW of the PSTH.

Barrel Cortex Neural Coding Patterns in Short-Term and Long-Term TBI

We first examined the effect of TBI on the 2 temporal measures of neural coding. In general, both complex stimuli produced similar results and so, while we describe the results from all 3 stimuli, only data for the trapezoid and object contact stimuli are illustrated throughout this article. These data can be viewed in the context of our previous work,[24,26] demonstrating that at 24 h and 4 d postsurgery, TBI results in a significant suppression of the maximum response rate (the PFR), with greatest suppression in upper cortical layers, and that this evolves into a suppression across all cortical layers, L2 to L5, at 2 wk postinjury[40] and, finally, into a relative hyperexcitability in supragranular layers at 8 to 10 wk postsurgery.[24,27] At the same time points, there were only inconsistent effects of TBI on the latency to that PFR.[24,26]

We now consider effects for the trapezoid stimulus in TBI + Veh animals compared to the Sham + Veh animals. Figure 2 shows mean RDM and HPW data in vehicle (peanut oil)-treated groups to each velocity of this stimulus at 4 d and at 8 wk postsurgery.

In the 4 d survival condition, there was no difference in the trapezoid-driven RDM (Fig. 2a, left column) between TBI and sham vehicle–treated groups, $F(1, 4)$ range = 0.01 to 2.83, P always > 0.05, in any layer and no significant interactions (P > 0.05). With respect to the HPW (Fig. 2b, left column), there were no group differences or interactions in L2, L4, or L5, $F(1, 4)$ range = 0.04 to 0.31, P always > 0.05, but it was significantly broader for sham animals in D3, $F(1, 4) = 7.56$, P < 0.01, and there was a significant interaction in U3, $F(4, 105) = 4.63$, P < 0.01; post hoc t tests revealed the HPW was significantly broader in TBI for the 30 mm/s ramp (P < 0.05) and significantly narrower at 150 and 250 mm/s (P < 0.05).

In the 8 wk survival condition, in TBI + Veh animals, RDM (Fig. 2a, right column) was significantly broader in U3, L4, and L5, $F(1, 4)$ range = 5.95 to 33.11, P always < 0.05, generally P < 0.01, but there were no group differences or interactions in L2 or D3, L2: $F(1, 4) = 3.61$, $P = 0.06$; D3: $F(1, 4) = 2.74$, $P = 0.10$. The HPW (Fig. 2b, right column)

was also significantly broader in TBI animals in L2 to L4, $F(1, 4)$ range = 4.57 to 37.57, P always < 0.05, but there was no significant difference in L5, $F(1, 4) = 3.54$, P > 0.05, or any significant interactions (P > 0.05).

We now consider the effects for complex stimuli, with object contact responses seen in Fig. 3. Overall, in the 4-d survival condition, there was no consistent effect of TBI on the RDM in any cortical layer: in response to the object contact stimulus (Fig. 3a, left column), significantly broader RDMs were recorded in TBI + Veh animals in L2 and U3, L2: $F(1, 9) = 3.96$, P < 0.05; U3: $F(1, 9) = 18.96$, P < 0.01, but not in any other layer (D3 to L5: $F(1, 9)$ range = 0.01 to 2.76 P always > 0.05, and no significant interaction in any layer (P > 0.05). In response to the rough surface stimulus (data not shown), compared to Sham + Veh animals, the RDMs in TBI + Veh animals were significantly narrower in L2 and L4, L2: $F(1, 9) = 22.74$, P < 0.01; L4: $F(1, 9) = 22.52$, P < 0.01, but there were no differences in U3, D3, or L5, $F(1, 9)$ range = 0.96 to 2.13, P always > 0.05, and no significant interactions in any layer (P > 0.05). With respect to the temporal bandwidth measure (Fig. 3b, left column), significantly broader HPWs were recorded from TBI animals in L2, U3, D3, and L5, $F(1, 9)$ range = 5.88 to 23.01, P always < 0.05, but not in L4, $F(1, 9) = 0.73$, P > 0.05, in response to the object contact stimulus and were significantly narrower in L2, U3, and L4, $F(1, 9)$ range = 9.98 to 35.45, P always < 0.01, in response to the rough surface stimulus (data not shown). There were no other group differences or interaction effects in any cortical layer (P > 0.05).

In the 8 wk survival condition, TBI consistently broadened the RDM and the HPW in response to both complex stimuli, in all cortical layers. Thus, for the object contact stimulus (Fig. 3a, right column), TBI + Veh animals had significantly broader RDMs in every cortical layer, $F(1, 9)$ range = 7.22 to 35.31, P always < 0.01, with no significant interactions (P > 0.05). Similarly, RDMs to the rough surface stimulus (data not shown) were also significantly broader in TBI animals in every cortical layer, $F(1, 9)$ range = 8.56 to 55.78, P always < 0.01. The temporal bandwidth, HPW, followed a similar pattern (Fig. 3b, right column), and for both complex stimuli (rough surface stimuli; data not shown), every cortical layer was broader in TBI + Veh animals than in Sham + Veh, $F(1, 9)$ range = 6.80 to 55.78, P always < 0.05.

In summary, at 4 d postinjury, there was no systematic difference in RDM or HPW in TBI + Veh animals compared to Sham + Veh animals. However, at 8 wk postinjury, there is a consistent broadening of the temporal response profile in U3, L4, and L5 in response to all stimuli and in all cortical layers in response to complex stimuli in TBI + Veh animals. Note, as described previously,[24,26] we have shown that at the short survival time point, TBI reduces PFRs in all cortical layers; the present analysis shows that when normalized for this change, there is no effect of TBI on response dispersion or temporal bandwidth; conversely, at the long survival time point, we have previously shown[24,27] that TBI increases PFRs in upper
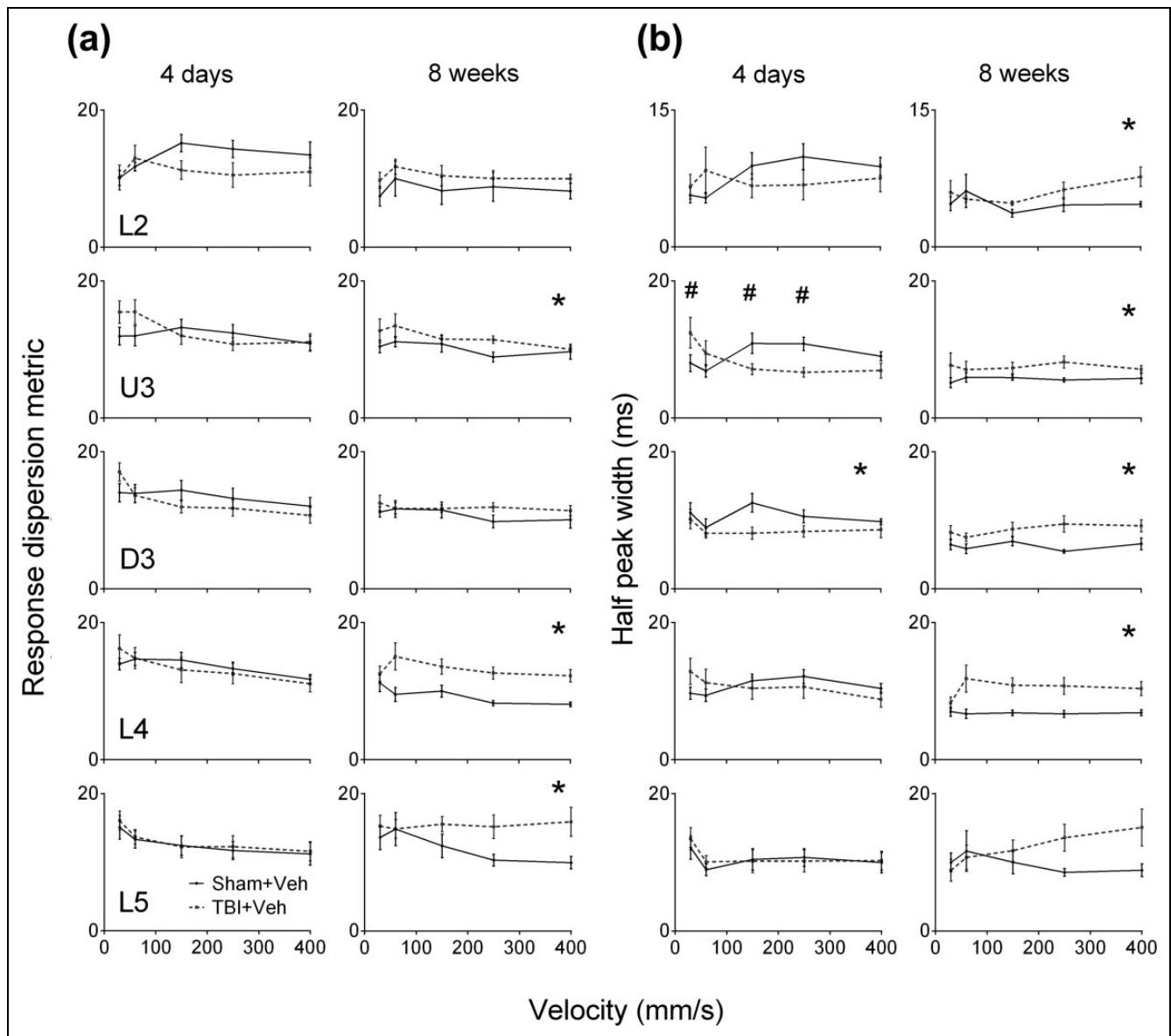
Figure 2. Comparison of the effect of traumatic brain injury (TBI) on temporal firing patterns in the short term and long term in the encoding of basic whisker deflections. (a) Mean response dispersion metric (RDM) responses were evoked by the trapezoid stimulus using 1 of the 5 ramp speed velocities in any of 5 cortical layers at either 4 d or 8 wk postsurgery. All comparisons represent mean multiunit responses recorded from both Sham + Vehicle (Veh) animals and TBI + Veh animals. Each of the columns below (a) shows RDMs to varying stimulus velocity, with the left column showing effects in the acute survival group and the right showing effects in the chronic survival group. Each of the columns below (b) shows half peak widths (HPWs) to varying stimulus velocity, with the left column showing effects in the acute survival groups and the right showing effects in the chronic survival group. Data are presented as mean ± standard error of the mean (SEM) and asterisks (*) indicate there was a significant layer-specific group difference in RDM or HPW (P < 0.05). The symbol "#" is result of a significant post hoc t test (P < 0.05).

cortical layers (only); when normalized for this change, in TBI, there is a broadening of the RDM and the temporal dispersion metric in all or almost all cortical layers.

P4 Sharpens Temporal Patterns in Middle Layers at Both 4 d and 8 wk Post-TBI

We next compared the effect of P4 treatment on responses in TBI animals compared to vehicle-treated TBI counterparts.

In brief, P4 consistently narrowed both temporal measures recorded in middle cortical layers from TBI animals in both the short term and long term, over which time period we have shown that maximum neural responsiveness (PFR) shift from hypoexcitability to hyperexcitability in supragranular in TBI animals.[24]

Figure 4 shows the mean RDM and HPW from the TBI groups at the 2 time points. In response to the trapezoid stimulus, in TBI + P4 animals, the RDM (Fig. 4a,
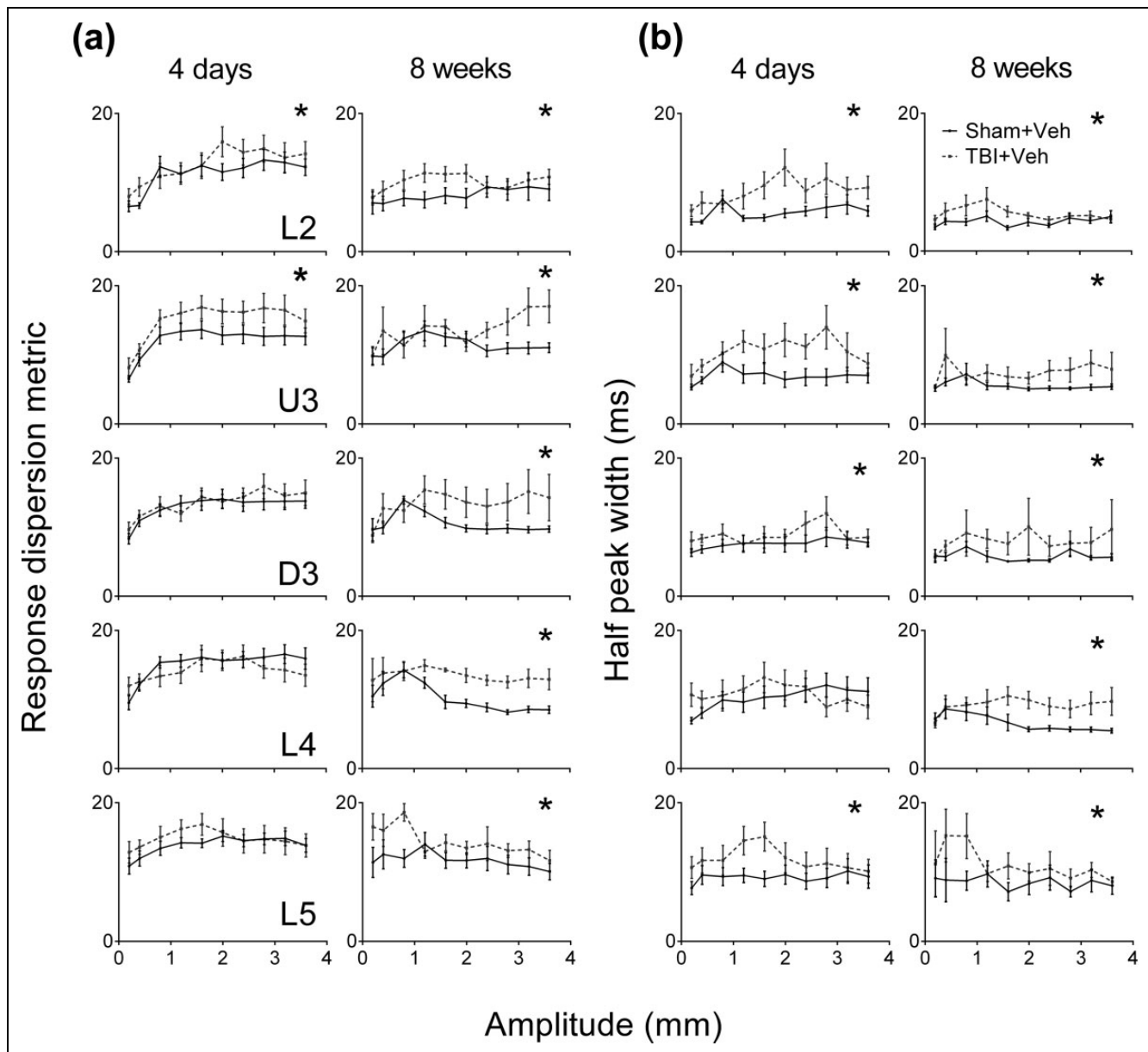
Figure 3. Comparison of the effect of traumatic brain injury (TBI) on temporal firing patterns in short term and long term in the encoding of complex whisker deflections. (a) Mean response dispersion metric (RDM) responses were evoked by the object contact stimulus using 1 of the 10 amplitudes in any of 5 cortical layers at either 4 d or 8 wk postsurgery. All comparisons represent mean multiunit responses recorded from both Sham + Vehicle (Veh) animals and TBI + Veh animals. Each of the columns below (a) shows RDMs to varying stimulus amplitude, with the left column showing effects in the acute survival groups and the right showing effects in the chronic survival groups. Each of the columns below (b) shows half peak widths (HPWs) to varying stimulus amplitude, with the left column showing effects in the acute survival groups and the right showing effects in the chronic survival group. Data are presented as mean ± standard error of the mean (SEM) and asterisks (*) indicate there was a significant layer-specific group difference in RDM or HPW ($P < 0.05$).

left column) at 4 d postinjury was significantly narrower in U3 to L4, $F(1, 4)$ range = 8.61 to 12.96, P always < 0.01, but not different in L2 or L5, L2: $F(1, 4) = 1.92$, $P > 0.05$; L5: $F(1, 4) = 3.46$, $P > 0.05$, nor were there any significant interactions ($P > 0.05$). The HPWs of the trapezoid-driven responses (Fig. 4b, left column) were significantly narrower in TBI + P4 animals in U3 to L5, $F(1, 4)$ range = 4.48 to 11.00, P always < 0.05, with

no significant group difference in L2 or any significant interactions ($P > 0.05$). At 8 wk postinjury, trapezoid-driven RDMs in TBI + P4 animals (Fig. 4a, right column) were significantly narrower in U3 to L5, $F(1, 4)$ range = 27.06 to 56.26, P always < 0.01, and there was no group difference in L2, $F(1, 4) = 3.91$, $P > 0.05$, and no significant interactions in any layer ($P > 0.05$). Exactly similar layer dependencies were seen with the HPW (Fig.
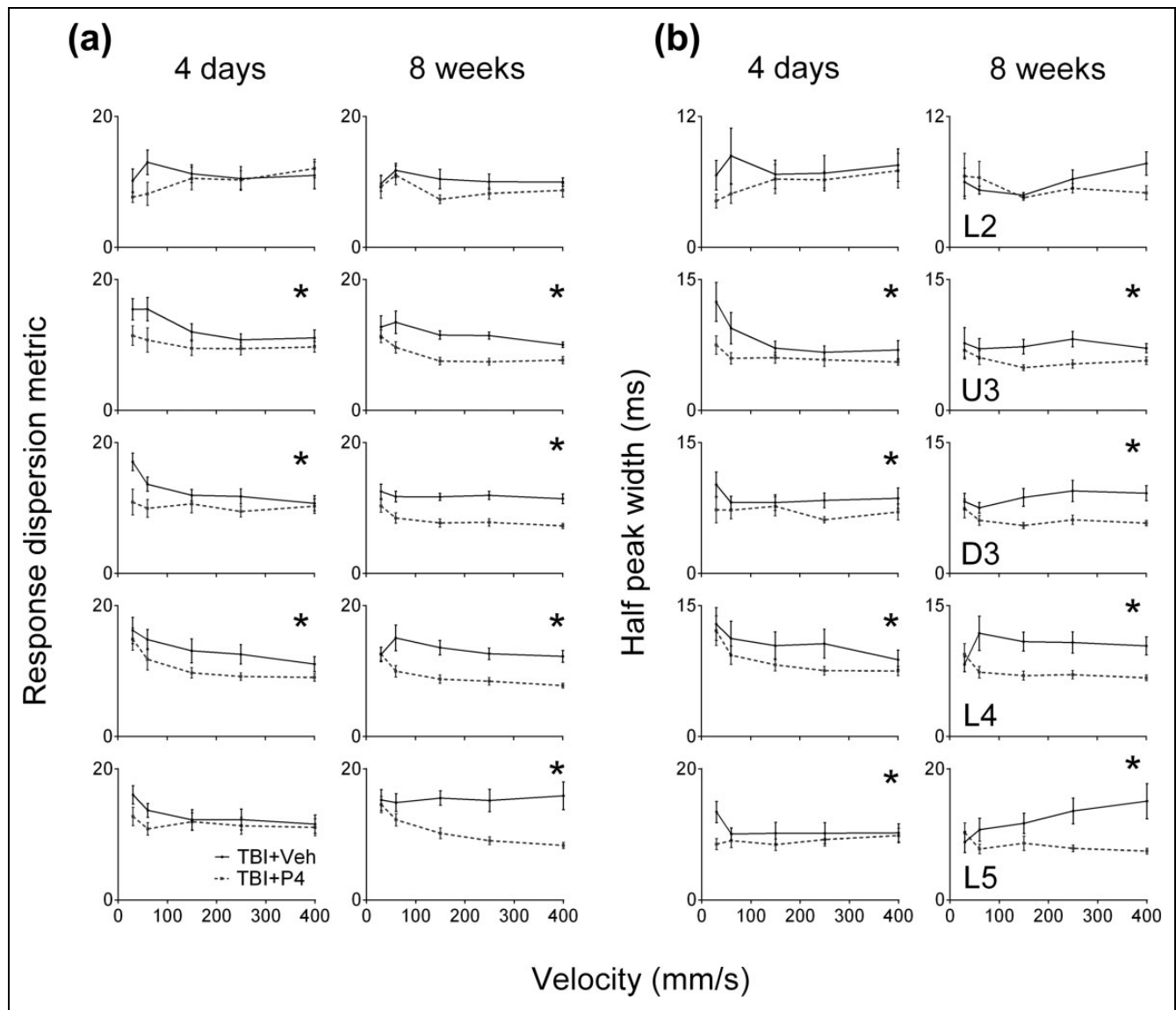
Figure 4. The effect of progesterone (P4) treatment on temporal coding properties in traumatic brain injury (TBI) animals in the short term and long term following basic whisker deflections. (a) Mean response dispersion metric (RDM) responses were evoked by the trapezoid stimulus using 1 of the 5 ramp speed velocities in any of the 5 cortical layers at either 4 d or 8 wk postsurgery. All comparisons represent mean multiunit responses recorded from both TBI + Vehicle (Veh) animals and TBI + P4 animals. Each of the columns below (a) shows RDMs to varying stimulus velocity, with the left column showing effects in the acute survival groups and the right showing effects in the chronic survival groups. Each of the columns below (b) shows half peak widths to varying stimulus velocity, with the left column showing effects in the acute survival groups and the right showing effects in the chronic survival group. Data are presented as mean ± standard error of the mean (SEM) and asterisks (*) indicate there was a significant layer-specific group difference ($P < 0.05$).

4b, right column) in TBI + P4 animals being significantly narrower in U3 to L5, $F(1, 4)$ range = 9.18 to 24.84, P always < 0.01, with no difference in L2 or any significant interactions in any layer ($P > 0.05$).

In response to the complex stimuli (of which object contact is presented in Fig. 5a, left column) in the 4 d TBI group, P4 significantly narrowed RDMs in all layers, object contact stimulus = L2 to L5: $F(1, 9)$ range = 15.57 to 34.23, P always < 0.01, or most layers (rough surface stimulus; data not shown): D3 to L5, $F(1, 9)$ range = 6.52 to 7.76, P always < 0.05; but not in L2, $F(1, 9) = 3.19$, $P > 0.05$, or U3,

$F(1, 9) = 3.53$, $P > 0.05$, and there were no significant interactions ($P > 0.05$). Similarly, the HPW in TBI + P4 animals (Fig. 5b, left column) was significantly narrower in all layers, object contact stimulus = L2 to L5: $F(1, 9)$ range = 16.24 to 29.04, P always < 0.01, or most layers (rough surface stimulus; data not shown): U3 to L5: $F(1, 9)$ range = 8.69 to 32.11, P always < 0.01. There were no other significant group effects or interactions in any layer ($P > 0.05$).

The effects in the 8 wk survival groups were very similar to those in the 4 d survival groups. Thus, in the TBI + P4 group, in response to the object contact stimulus (Fig. 5a,
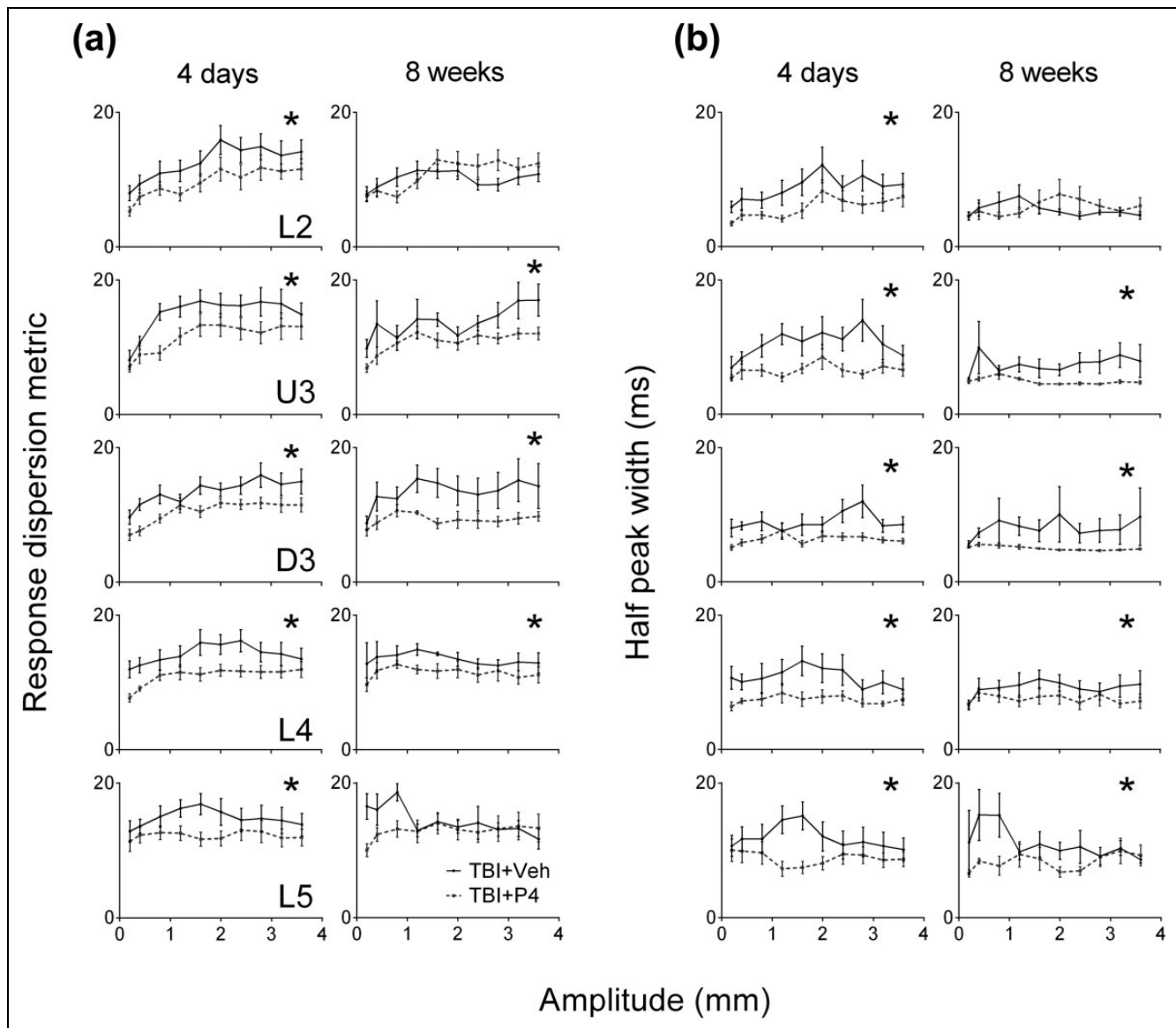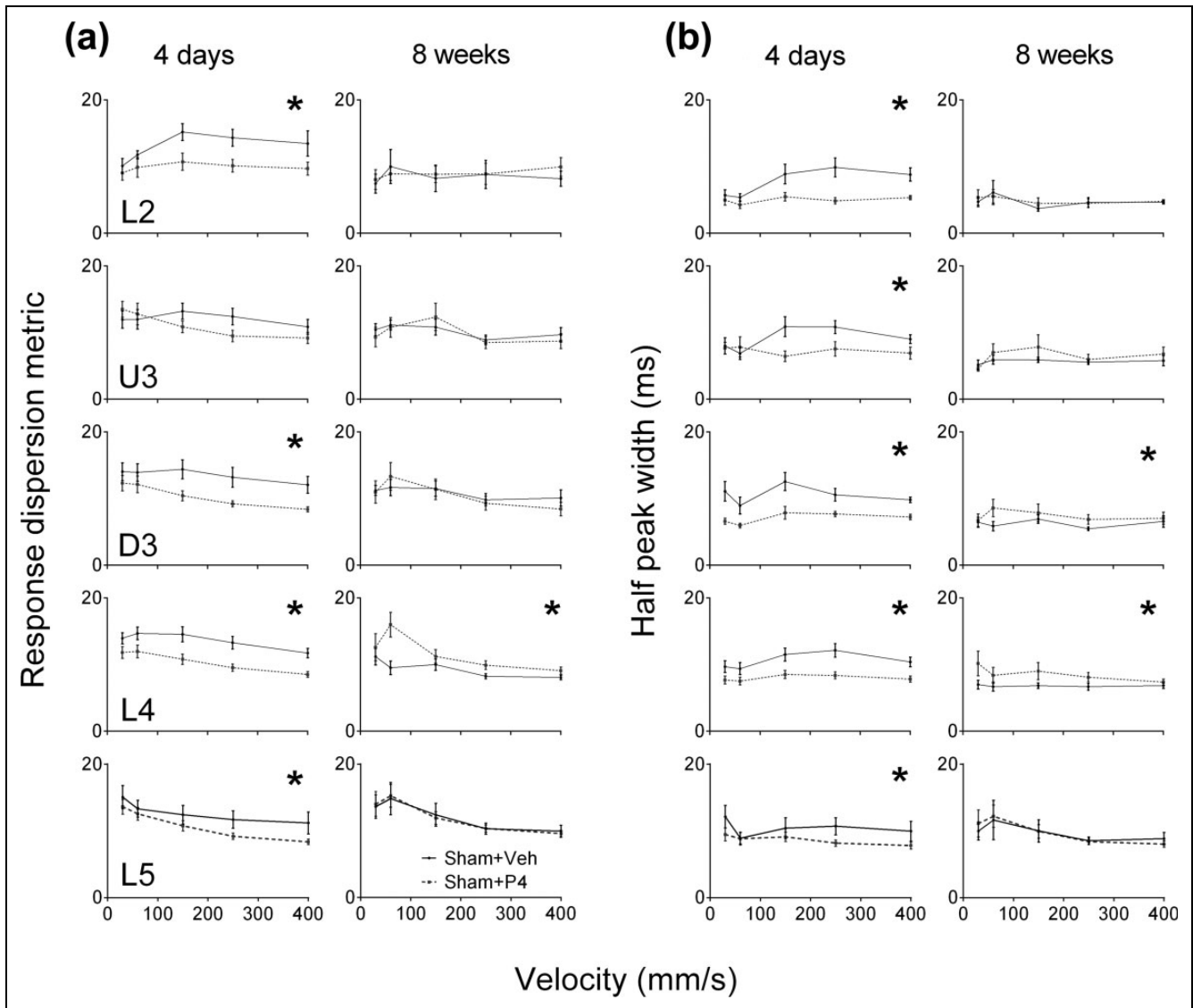
Figure 5. The effect of progesterone (P4) treatment on temporal coding properties in traumatic brain injury (TBI) animals in short term and long term following complex whisker deflections. (a) Mean response dispersion metric (RDM) responses were evoked by the object contact stimulus using 1 of the 10 amplitudes in any of the 5 cortical layers at either 4 d or 8 wk postsurgery. All comparisons represent mean multiunit responses recorded from both TBI + Vehicle (Veh) animals and TBI + P4 animals. Each of the columns below (a) shows RDMs to varying stimulus amplitude, with the left column showing effects in the acute survival groups and the right showing effects in the chronic survival groups. Each of the columns below (b) shows half peak widths to varying amplitude, with the left column showing effects in the acute survival groups and the right showing effects in the chronic survival groups. Data are presented as mean $\pm$ standard error of the mean and asterisks (*) indicate there was a significant layer-specific group difference ($P < 0.05$).

right column), there were significantly narrower RDMs in U3 to L4, $F(1, 9)$ range = 11.03 to 19.23, P always < 0.01, but similar RDMs in L2 and L5, L2: $F(1, 9) = 1.47, P > 0.05$; L5: $F(1, 9) = 3.79, P > 0.05$, with no significant interactions in any layer ($P > 0.05$). Similarly, with the rough surface stimulus (data not shown), TBI + P4 animals had significantly narrower RDMs in L2 to L5, $F(1, 9)$ range = 7.83 to 47.94, P always < 0.01, with no interactions in any layer ($P > 0.05$). The HPWs (Fig. 5b, right column) recorded to both complex stimuli were significantly narrower in TBI +

P4 animals than in TBI + Veh animals in U3 to L5, $F(1, 9)$ range = 7.90 to 30.91, P always < 0.01. There were no other group effects or any interactions ($P > 0.05$).

In summary, at 4 d postinjury across the suite of simple and complex stimuli, TBI + P4 animals consistently had narrower RDMs in D3 and L4 and narrower HPWs in D3 to L5 and, at 8 wk postinjury, consistently narrower RDMs and HPWs at least in U3 to L4 and for the rough surface stimulus also in L2 and L5. Thus, at both postsurgery time points, P4 narrowed temporal firing patterns in middle

Figure 6. The effect of progesterone (P4) treatment on temporal coding properties in sham animals in the short term and long term following basic whisker deflections. (a) Mean response dispersion metric (RDM) responses were evoked by the trapezoid stimulus using 1 of the 5 ramp speed velocities in any of the 5 cortical layers at either 4 d or 8 wk postsurgery. All comparisons represent mean multiunit responses recorded from both Sham + Vehicle (Veh) animals and Sham + P4 animals. Each of the columns below (a) shows RDMs to varying stimulus velocity, with the left column showing effects in the acute survival groups and the right showing effects in the chronic survival groups. Each of the columns below (b) shows half peak widths to varying stimulus velocity, with the left column showing effects in the acute survival groups and the right showing effects in the chronic survival group. Data are presented as mean $\pm$ standard error of the mean (SEM) and asterisks (*) indicate there was a significant layer-specific group difference ($P < 0.05$).

layers in the injured brain, consistent with a role in promoting inhibition.

P4 Narrows Temporal Profiles in Sham Animals at 4 d but Not at 8 wk Postsurgery

Finally, we analyzed the effect of P4 on the responses of animals that underwent sham surgery. In brief, we show that, with respect to the temporal aspects of responses, P4 narrows RDM but not HPW in sham animals in the short term, consistent with it causing a decrease in PFR, putatively by

promoting inhibition, but has no systematic effect on long-term temporal aspects of firing patterns.

Figure 6 shows the mean RDM and HPW of responses recorded in Sham + Veh and Sham + P4 animals to the trapezoid stimulus at 4 d (left columns) and 8 wk (right columns) postsurgery. At 4 d postsurgery, the P4-treated animals had significantly narrower RDMs (Fig. 6a, left columns) in all cortical layers except U3, L2, D3 to L5: $F(1, 4)$ range = 6.82 to 35.05, P always < 0.05. There was no group difference in U3 or any interaction effects ($P > 0.05$). Similarly, the HPW (Fig. 6b, left column) from Sham + P4
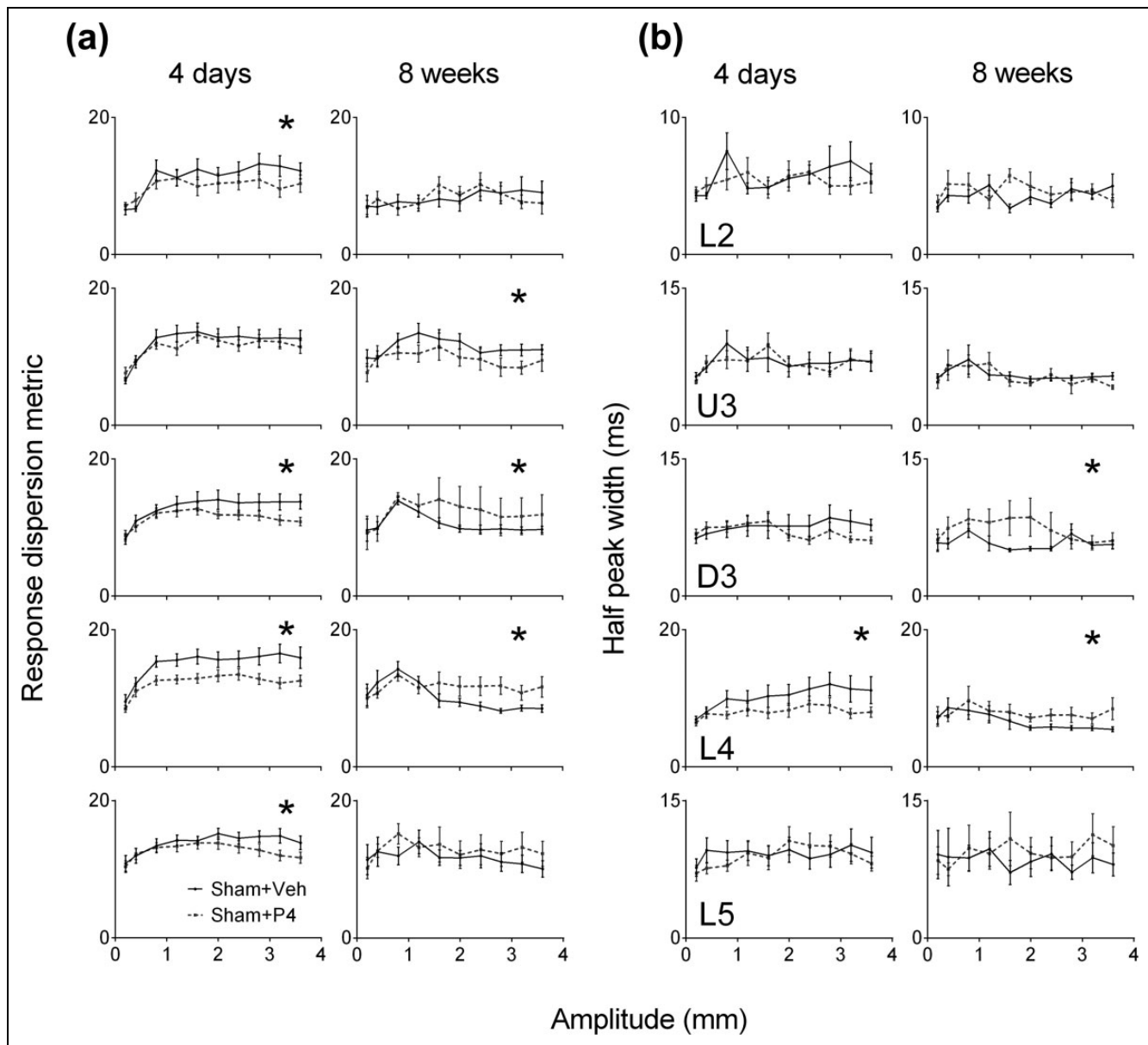
Figure 7. The effect of progesterone (P4) treatment on temporal coding properties in sham animals in short term and long term following complex whisker deflections. (a) Mean response dispersion metric (RDM) responses were evoked by the object contact stimulus using 1 of the 10 stimulus amplitudes in any of the 5 cortical layers at either 4 d or 8 wk postsurgery. All comparisons represent mean multi-unit responses recorded from both Sham + Vehicle (Veh) animals and Sham + P4 animals. Each of the columns below (a) shows RDMs to varying stimulus amplitude, with the left column showing effects in the acute survival groups and the right showing effects in the chronic survival groups. Each of the columns below (b) shows half peak widths to varying stimulus amplitude, with the left column showing effects in the acute survival groups and the right showing effects in the chronic survival group. Data are presented as mean $\pm$ standard error of the mean (SEM) and asterisks (*) indicate there was a significant layer-specific group difference ($P < 0.05$).

animals was significantly narrower in L2 to L5, $F(1, 4)$ range = 6.94 to 44.03, P always < 0.01, with no interactions in any layer ($P > 0.05$). At 8 wk postinjury, only in L4, $F(1, 4) = 10.70$, $P < 0.01$, there was significant broadening of the trapezoid-driven RDM (Fig. 6a, right column) in Sham + P4 animals compared to Sham + Veh animals, while in all other layers, there was no difference, $F(1, 4)$ range <0.01 to 0.13, P always > 0.05; no interactions, $P > 0.05$). The HPW in Sham + P4 animals was significantly broader only in D3

and L4, D3: $F(1, 4) = 4.34$, $P < 0.05$; L4: $F(1, 4) = 9.20$, $P < 0.01$, and there were no other interactions ($P > 0.05$).

Figure 7 shows mean RDM and HPW of responses recorded from Sham + Veh and Sham + P4 animals to the object contact stimulus at 4 d and 8 wk postsurgery. In the short-term groups, Sham + P4 animals always had narrower RDMs in L2, D3, L4, and L5 than their vehicle-treated counterparts, object contact stimulus (Fig. 7a, left column): $F(1, 9)$ range = 5.15 to 40.69, P always < 0.05; rough

surface stimulus (data not shown): $F(1, 9)$ range = 6.01 to 36.17, P always < 0.05. There were no group differences in U3 in response to either stimulus ($P > 0.05$) and no significant interactions ($P > 0.05$). The HPW was significantly narrower in L4, $F(1, 9) = 16.68$, $P < 0.05$, following the object contact stimulus (Fig. 7b, left column) and in all cortical layers, $F(1, 9)$ range = 13.39 to 54.69, P always < 0.01, following the rough surface stimulus (data not shown), with no other group or interaction effects for either complex stimulus ($P > 0.05$).

At 8 wk postsurgery, in response to the object contact stimulus (Fig. 7a, right column), effects were inconsistent: Sham + P4 RDM was significantly narrower than Sham + Veh responses in U3, $F(1, 9) = 9.81$, $P < 0.01$, significantly broader in D3 and L4, D3: $F(1, 9) = 6.93$, $P < 0.01$; L4: $F(1, 9) = 7.17$, $P < 0.01$, and there were no group differences in L2 or L5, L2: $F(1, 9) < 0.01$, $P > 0.05$; L5: $F(1, 9) = 1.88$, $P > 0.05$, or interactions in any layer ($P > 0.05$). Similar inconsistent effects were seen in response to the rough surface stimulus (data not shown): Sham + P4 animal RDMs were significantly narrower in U3 and L5, U3: $F(1, 9) = 26.26$, $P < 0.01$; L5: $F(1, 9) = 16.05$, $P < 0.01$, but not different in L2, D3 or L4, $F(1, 9)$ range <0.01 to 1.05, P always > 0.05, and there were no interaction effects ($P > 0.05$). The HPWs in response to the complex stimuli were similarly varied with Sham + P4 responses being significantly broader than those in Sham + Veh animals in D3 and L4, D3: $F(1, 9) = 11.56$, $P < 0.01$; L4: $F(1, 9) = 6.18$, $P < 0.05$, for the object contact stimulus (Fig. 7b) and significantly narrower in U3 and L5, U3: $F(1, 9) = 11.39$, $P < 0.01$; L5: $F(1, 9) = 9.24$, $P < 0.01$, in response to the rough surface stimulus (data not shown). There were no other group or interaction effects ($P > 0.05$).

In summary, P4 consistently narrows the response profile of sham animals compared to vehicle-treated animals in the short term; but in the long term, P4 broadened RDM in L4 while sharpening it in U3 and broadened the HPW in L4 and D3 but only to the trapezoid and object contact stimuli. Thus, the long-term change was not systematic, and it is difficult to draw conclusions about P4 long-term effects on the uninjured brain.

P4 Alters TBI and Sham Animal RDM and HPW Differently in the Long Term

We have previously shown that at 4 d postsurgery, P4 decreases the PFR proportionately in sham and TBI animals. However, at 8 wk postsurgery, P4 increases PFR in sham animals, while, in TBI animals, it increases PFR in L2 and decreases it in U3.[24] Hence, we now compare the RDM and HPW at both time points between P4-treated sham and TBI animals. In general, P4 narrows the temporal features of response patterns in sham and TBI animals proportionately at 4 d postsurgery consistent with promoting inhibition in both groups. However, as we describe below, in the long term, it does not alter the temporal features of

responses in sham animals but does alter them in TBI animals, suggesting a loss of TBI capacity to adapt to sustained P4 treatment.

Figure 1 shows the mean RDM and HPW in Sham + P4 and TBI + P4 animals to the trapezoid stimulus at 4 d (left columns) and 8 wk (right columns) postsurgery. At 4 d postsurgery (Fig. 8a, left), RDM was not significantly different between Sham + P4 and TBI + P4 animals, and there were no interaction effects ($P > 0.05$). Similarly, there were no significant group differences in HPW (Fig. 8b, left) at 4 d postsurgery in L2, U3, D3, or L5 ($P > 0.05$). There was a significant interaction in L4, $F(4, 130) = 4.12$, $P < 0.01$, due to a significant group difference when the ramp speed was 30 mm/s ($P < 0.01$) but not at any other velocity (post hoc t tests, $P > 0.05$).

At 8 wk postsurgery, the trapezoid-driven RDM (Fig. 8a, right) was significantly narrower in TBI animals in D3 and L4, D3: $F(1, 4) = 12.16$, $P < 0.01$; L4: $F(1, 4) = 11.42$, $P < 0.01$, but not different in any other layer, and there were no interaction effects ($P > 0.05$). The HPW (Fig. 8b, right) was significantly narrower in TBI + P4 animals in D3 and L5, D3: $F(1, 4) = 5.63$, $P < 0.05$; L5: $F(1, 4) = 4.41$, $P < 0.05$, but not in any other layer, and there were no interaction effects ($P > 0.05$).

With respect to the metrics for the responses to complex stimuli, at 4 d postsurgery, RDM to the object contact stimulus (Fig. 9a, left column) was significantly narrower in TBI + P4 animals than in Sham + P4 counterparts in D3 and L4, D3: $F(1, 9) = 7.04$, $P < 0.01$; L4: $F(1, 9) = 12.40$, $P < 0.01$. Relatively similar effects were seen to the rough surface stimulus (data not shown). The HPW was significantly narrower in D3, $F(1, 9) = 5.57$, $P < 0.05$, in Sham + P4 animals following the object contact stimulus (Fig. 9b, left) and also to the rough surface stimulus (data not shown) where it was also significantly broader in L2, $F(1, 9) = 6.41$, $P < 0.05$. There were no other significant group differences or interactions in any layers following either complex stimulus ($P > 0.05$).

At 8 wk postsurgery (Fig. 9a right), the RDM to the object contact stimulus in TBI + P4 animals was significantly broader in U3, $F(1, 9) = 4.33$, $P < 0.05$, and significantly narrower in D3, $F(1, 9) = 15.38$, $P < 0.01$, but not different in any other layer, and there were no interactions ($P > 0.05$). Again similar effects were found to the rough surface stimulus (data not shown). A summary of the effects of P4 on response profiles of both TBI and sham animals can be seen in the schematic diagram below.

To compare the effects of P4 on RDM and HPW between sham and TBI groups, we calculated the normalized ratio of RDM and the HPW using lamina-specific group means as:

(i) TBI condition: (TBI + P4 − TBI-Veh)/(TBI + P4 + TBI-Veh)
(ii) Sham surgery condition: (Sham-P4 − Sham-Veh)/(Sham-P4 + Sham-Veh).
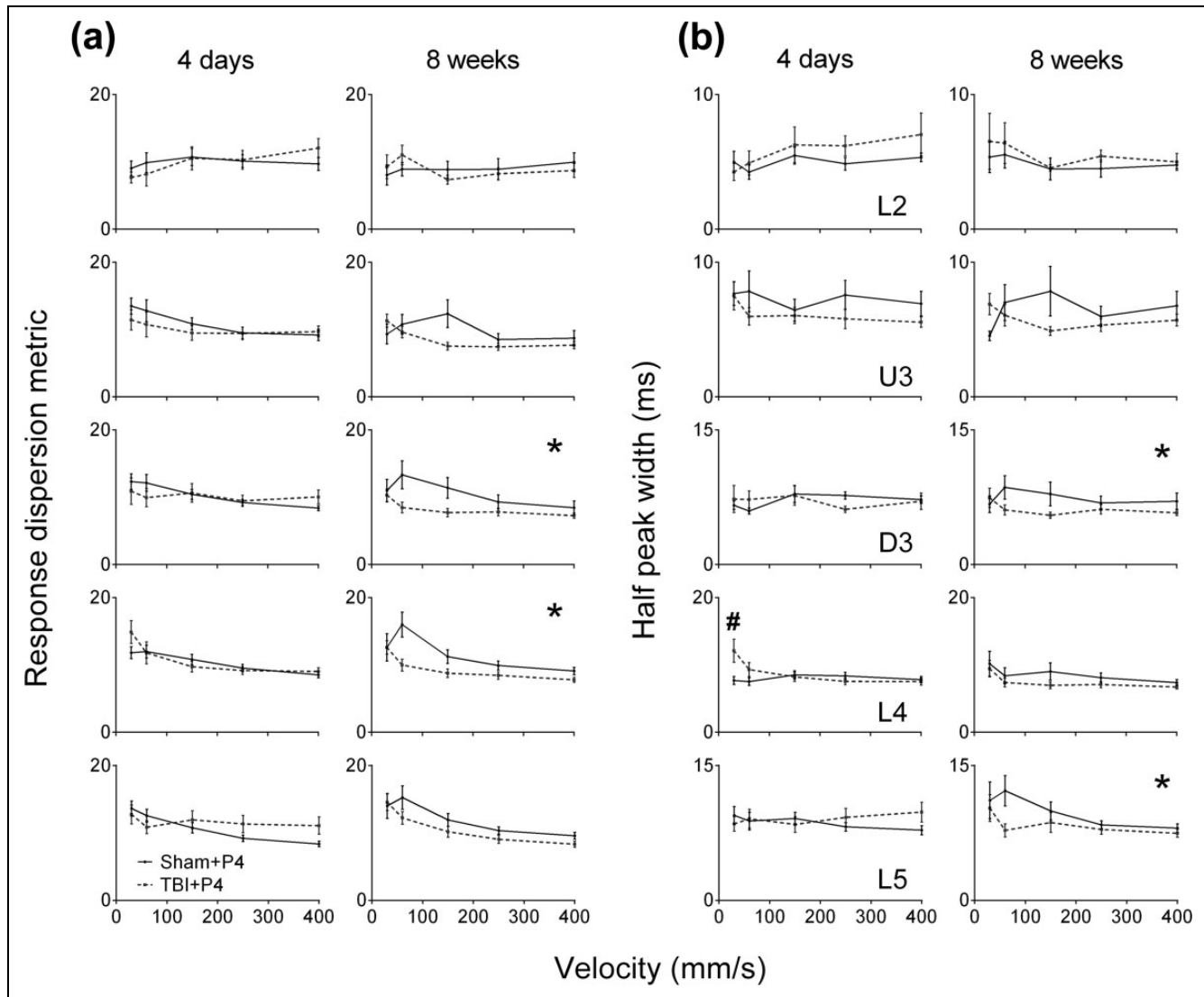
Figure 8. The effect of traumatic brain injury (TBI) on temporal coding properties in progesterone (P4)-treated animals in short term and long term following basic whisker deflections. (a) Mean response dispersion metric (RDM) responses were evoked by the trapezoid stimulus using 1 of the 5 ramp speed velocities in any of the 5 cortical layers at either 4 d or 8 wk postsurgery. All comparisons represent mean multiunit responses recorded from both Sham + P4 animals and TBI + P4 animals. Each of the columns below (a) shows RDMs to varying stimulus velocity, with the left column showing effects in the acute survival groups and the right showing effects in the chronic survival groups. Each of the columns below (b) shows half peak widths to varying stimulus velocity, with the left column showing effects in the acute survival groups and the right showing effects in the chronic survival group. Data are presented as mean ± standard error of the mean (SEM) and asterisks (*) indicate there was a significant layer-specific group difference ($P < 0.05$). The symbol "#" is result of a significant post hoc t test ($P < 0.05$).

These ratios, which can range from +1.0 (P4 treatment broadens temporal firing patterns compared to the Veh-treated group for that surgical condition) to −1.0 (P4 treatment narrows temporal firing patterns compared to the Veh-treated group for that surgical condition), are shown in Fig. 10 for the RDM and Fig. 11 for the HPW.

These figures illustrate that for both surgical groups, in the short term, P4 treatment narrowed temporal responses in D3 to L5, and this was proportionately similar in the 2 treatment groups. However, at 8 wk postsurgery, P4 treatment appeared to have little to no effect on the broadness of sham responses

but narrowed the responses in TBI animals in layers, D3, L4, and L5. In all other layers, the lack of effect tended to be very similar in the 2 surgical conditions in the long term.

Summary of Results

We have summarized the findings of this article schematically to highlight and simplify the 3 main effects we have reported above. Figure 12a shows the waveforms of the 3 whisker protraction stimuli we used to elicit neural activity in the barrel cortex. In Fig. 12b, we see the main effects we report above in
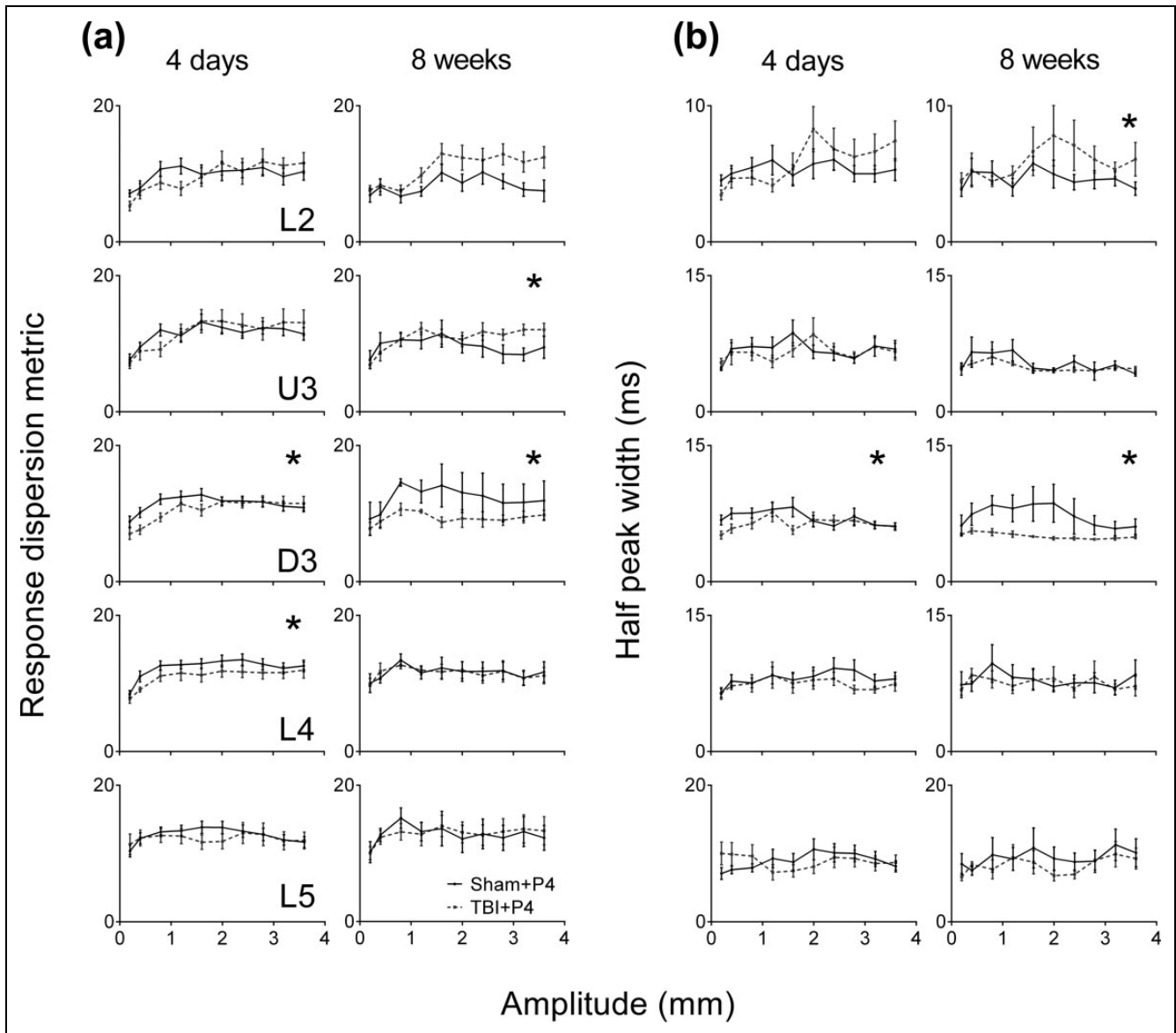
Figure 9. The effect of traumatic brain injury (TBI) on temporal coding properties in progesterone (P4)-treated animals in short term and long term following complex whisker deflections. (a) Mean response dispersion metric (RDM) responses were evoked by the object contact stimulus using 1 of the 10 stimulus amplitudes in any of the 5 cortical layers at either 4 d or 8 wk postsurgery. All comparisons represent mean multiunit responses recorded from both Sham + P4 animals and TBI + P4 animals. Each of the columns below (a) shows RDMs to varying stimulus amplitude, with the left column showing effects in the acute survival groups and the right showing effects in the chronic survival groups. Each of the columns below (b) shows half peak widths to varying stimulus amplitude, with the left column showing effects in the acute survival groups and the right showing effects in the chronic survival group. Data are presented as mean $\pm$ standard error of the mean (SEM) and asterisks (*) indicate there was a significant layer-specific group difference ($P < 0.05$).

the short-term postsurgery cohort: that there is no short-term change in the response profile as a function of TBI alone, that P4 treatment narrows the response profile of TBI animals in all layers other than L2, and that P4 treatment has a similar, if less consistent effect on the response profile of sham-treated animals. All short-term group comparisons reported above in the form of 2-way ANOVA are detailed in Fig. 12 to concisely summarize data and to present data in a manner in which the relevant findings are easily identifiable. In Fig. 12c, we present the long-term comparison of both

TBI and P4 treatment in which TBI broadens the response profile in all cortical layers, P4 narrows the response profile in all cortical layers other than L2 in TBI animals, and P4 has no long-term effect on sham animals.

Discussion

Previously, we have shown that P4 treatment influences diffuse TBI-induced changes in barrel cortex firing strength in short-term post-TBI but not in long-term post-TBI.[24]
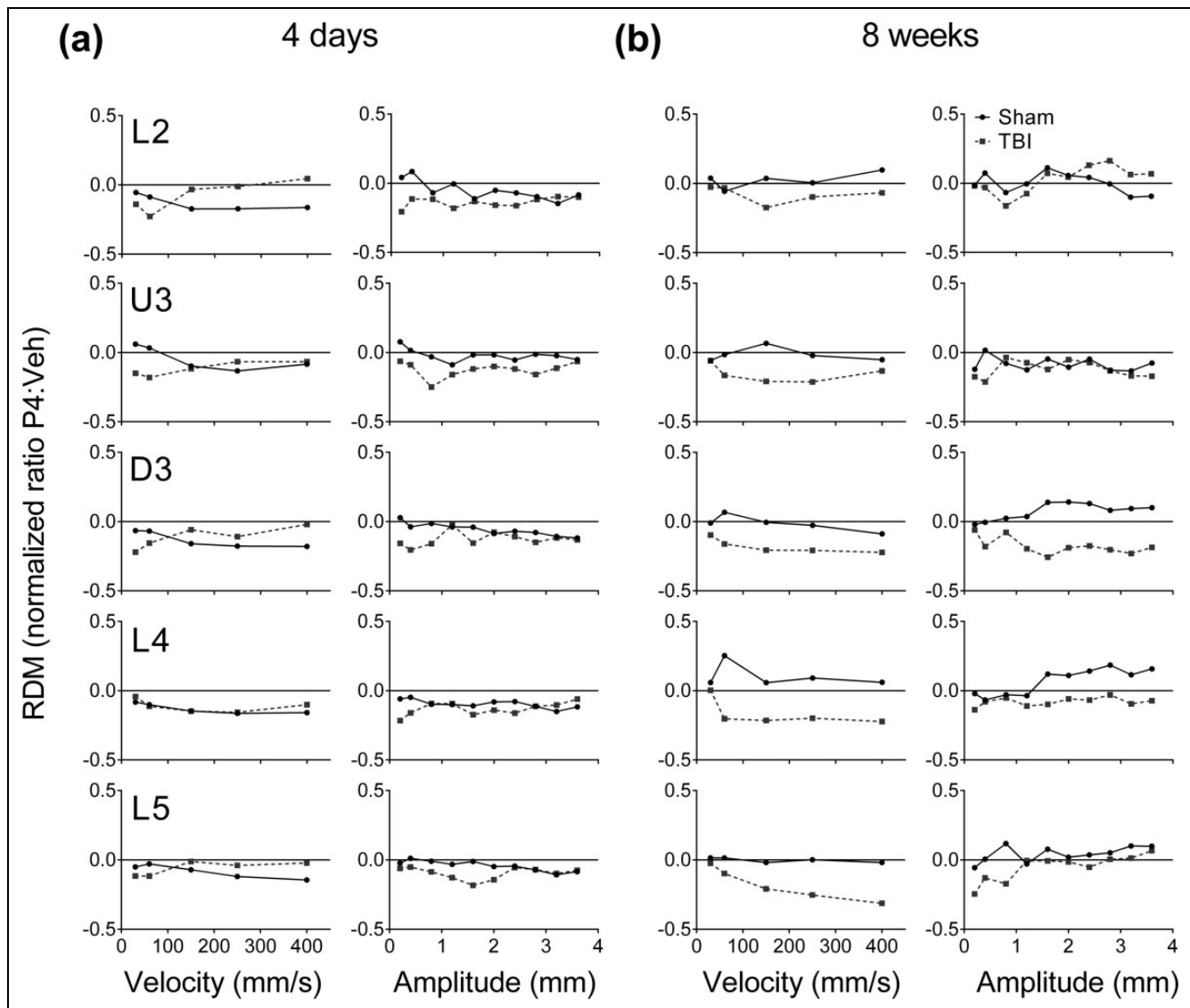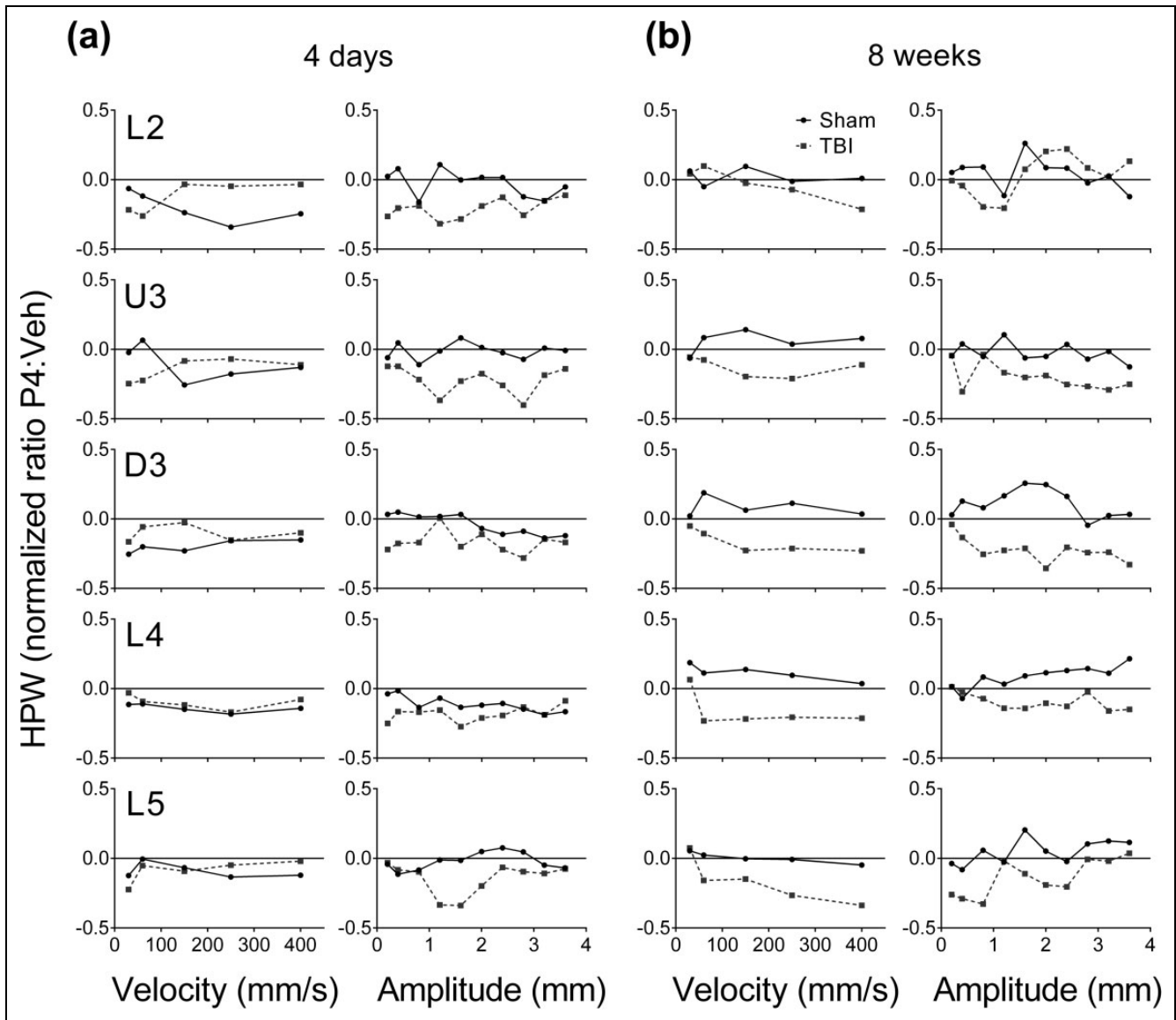
Figure 10. Ratio of response dispersion metric (RDM) narrowing as a function of progesterone (P4) in sham and traumatic brain injury (TBI) animals. Normalized RDM ratios in injured and uninjured brains are shown for (a) trapezoid and (b) object contact evoked responses and were calculated using lamina-specific RDMs as follows: for in the TBI condition (TBI-P4 − TBI-Vehicle (Veh))/(TBI-P4 + TBI-Veh) and for the sham surgery condition as (Sham-P4 − Sham-Veh) / (Sham-P4 + Sham-Veh). The normalized ratio ranges from +1.0 (P4 treatment broadens response patterns compared to the vehicle-treated group for that surgical condition) to −1.0 (P4 treatment narrows response patterns compared to the vehicle-treated group for that surgical condition).

Specifically, we found that (1) in the short term (24 h or 4 d post-TBI), diffuse brain injury resulted in reductions in PFRs to variations in stimulus velocity (simple trapezoidal stimuli to the whiskers) or in stimulus amplitude (using complex stimuli) in upper-to-middle cortical layers, L2 to L4.[24,26] (2) In the short-term case (tested at 4 d post-TBI), P4 treatment further exacerbated the TBI-induced reduction in neuronal PFRs in the uppermost cortical layers, L2 and U3, and suppressed spontaneous activity.[24] (3) In the long term (8 wk post-TBI), diffuse TBI in itself caused hyperexcitation in neuronal PFRs but only in supragranular cortical layers.[24,27] (4) In the long-term case, P4 treatment had no effect on the TBI-induced increased supragranular

firing rates or on the recovered normal firing rates in the other cortical layers.[24]

The P4 effects we reported in TBI were comparable (short term) or contrasting (long term) to effects in sham animals: suppression of response strength occurred with short-term P4 treatment, but there was no long-term effect in the TBI brain. Overall, in neither short-term nor long-term diffuse TBI did P4 provide any apparent benefits to sensory cortical neuronal responses when indexed in the PFRs. However, as we noted in the Introduction, sensory encoding is defined also by the temporal patterns of neuronal firing profiles and so we have now reexamined our previous data using new neuronal response metrics based

Figure 11. Ratio of half peak width (HPW) narrowing as a function of progesterone (P4) in sham and traumatic brain injury (TBI) animals. Normalized HPW ratios in injured and uninjured brains are shown for (a) trapezoid and (b) object contact evoked responses and were calculated using lamina-specific HPWs as follows: for the TBI condition (TBI-P4 − TBI-Veh) / (TBI-P4 + TBI-Veh) and for the sham surgery condition as (Sham-P4 − Sham-Veh) / (Sham-P4 + Sham-Veh). The normalized ratio ranges from +1.0 (P4 treatment broadens response patterns compared to the vehicle-treated group for that surgical condition) to −1.0 (P4 treatment narrows response patterns compared to the vehicle-treated group for that surgical condition).

on the temporal patterns of responses. We extracted the RDM (the normalized response strength across the stimulus duration), the half-peak width, and the temporal bandwidth of the response profile across the stimulus duration. Note that normalizing the first metric to the PFR prevents interference of this metric by the above-noted changes in PFRs in short-term and long-term TBI,[24,26,27] while the second measure is already independent of the PFR. With these metrics of the temporal features of neuronal responses, we found that (see Fig. 12) (1) in the short term, TBI did not alter the response dispersion across the

cortical column, but in the long term, it broadened the response profiles in all cortical layers; (2) in both the short term and the long term, P4 sharpened the response profile in TBI animals in all layers other than L2; (3) P4 also sharpened the response profile in sham animals in all layers in the short term but had no effect on the response profile in the long term.

These results indicate that there may be a benefit of P4 in the encoding and thus the sensation of tactile stimuli; and if this is transferable to the clinic, it would make for positive patient outcomes.
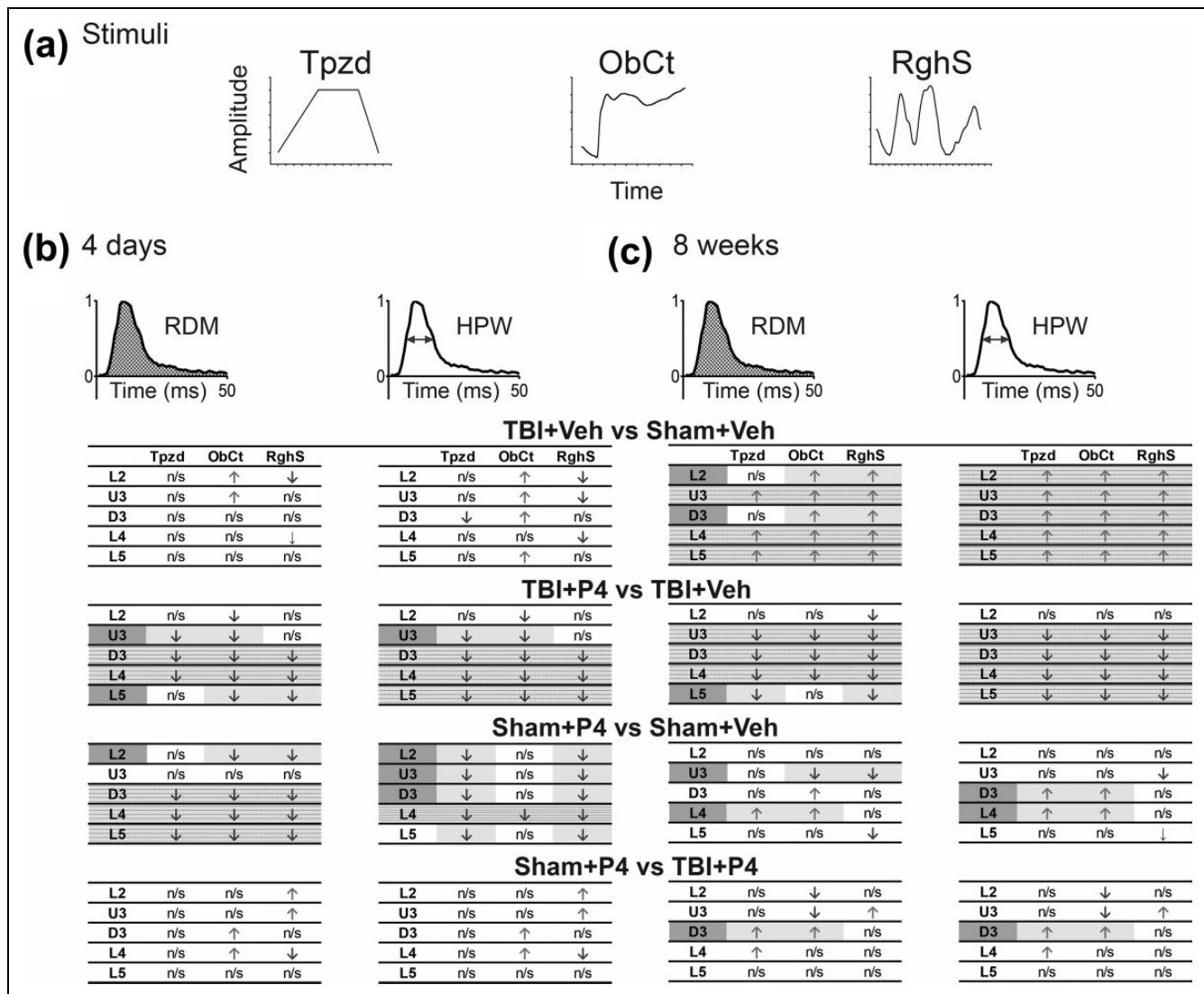
Figure 12. Schematic summary of the presented stimuli and all group comparisons. (a) Waveforms of the stimuli used to evoke whisker-driven neural activity. As detailed in the Materials and Methods section, we applied 1 of the 5 ramp speeds when presenting the trapezoid stimulus (Tpzd) and 1 of the 10 amplitudes when presenting either the object contact (ObCt) or rough surface (RghS) stimuli. (b) Schematic examples of how both the response dispersion metric and half peak width were calculated from normalized peristimulus time histograms constructed from each multiunit cluster. Also shown are tabular results of each of the comparisons we made (e.g., traumatic brain injury [TBI] + vehicle [Veh] vs. Sham + Veh) in which an "up" arrow indicates the first group of the comparison produced a significantly broader response and a "down" arrow indicates a significantly narrower response at 4 d postsurgery across all cortical layers for both TBI and sham animals. (c) Long-term responses are tabulated using the same model as that used for short-term results with "up" arrows indicating a broader response profile and "down" narrower comparisons for the first group mentioned. In both (b) and (c), where a group response profile change was consistent across stimuli, individual comparisons are shaded in light gray; if the cortical layer label is shaded in dark gray, the response change was only consistent for 2 of the 3 stimuli; and if the cortical layer label and corresponding row contain texture, all stimuli evoked the same change in that layer for that particular comparison.

Short-Term Effects of P4 on Response Profiles in the Injured and Uninjured Brain

TBI initiates a series of pathophysiological molecular and structural changes that begin with impact and can persist into the long term.[41,42] TBI-induced pathophysiology results in immediate and short-term hypo-excitation in neuronal response rates in upper-to-middle cortical layers,[24,26] possibly due to stress wave phenomena, damage to endothelial cells in the BBB, an increase in intracranial pressure, alterations in the ionic balance,[42–45] and by a wave of cortical spreading depression which causes widespread hyperpolarization and long-term presynaptic depression.[33] We now show that the short-term changes in firing rate are not accompanied by any systematic changes in the temporal response properties. Thus, the neural mechanisms that control for temporal precision in cortex, such as L4 neurogliaform activation as well as temporally precise thalamocortical

input,[46] appear to be unaffected by TBI in the early postinjury period.

Treatment with P4 over the same short time further suppresses upper cortical PFRs in the TBI by amounts proportionate to effects on L2 and U3 PFR in sham animals[24] and also sharpens the temporal response profile in TBI and sham animals (present study). P4 easily crosses the BBB and activates inhibitory interneurons and limits excitatory activity[47–53] by gamma-aminobutyric acid (GABA)-mediated inhibition and by reducing the excitability of kainate receptors,[54–57] thereby attenuating and modulating neural responses. These effects could account for the short-term PFR reductions reported previously and response profile sharpening in sham and TBI brains that we report here.

P4 receptors are found in every neuron type in the brain[53] and different GABAergic interneurons act to attenuate response strength, sharpen the response profile, and decrease sensitivity to incoming synaptic activity.[58–60] Yet, our results show that P4 had no sharpening effect in L2 in the TBI brain, suggesting that the inhibitory coding mechanism that is activated by P4 treatment in sham animals, that is not associated with response strength, is inactive in L2 in TBI animals. Between 6 h and 10 d post-TBI, there is a downregulation of thalamic GABA receptors which persists for up to 4 mo postinjury.[61] The fact that, despite this loss of GABA receptors in the TBI thalamus, we see similar response profile sharpening in sham and TBI animals, other than in L2 in TBI brains, must indicate that P4 is exerting effects in cortex not thalamus. We have shown that there is loss of calretinin expressing interneurons in the upper cortical layers long-term postinjury[16] and work is underway to determine whether these interneurons are affected as early as 4 d post-TBI; if so, it could account for the absence of P4 narrowing the response profile in L2 (which contains the greatest density of GABAergic neurons[62]) in the TBI animal. L2 codes for complex stimulus elements,[63] and a lack of P4 effect on the response profile in this layer may also mean that elements of complex sensory perception are not benefited by P4 administration.

In general, these results support our previous conclusions that GABAergic inhibitory mechanisms are still viable in the cortex in the short term following TBI.[24] However, other factors cannot be discounted. We have previously argued that ionic imbalance may also be a factor in supragranular hypo-excitation seen in the immediate aftermath of TBI,[26] and this could still be present at 4 d postinjury and cause excitatory neuron dysfunction. For example, in the visual cortex during the presentation of high-contrast stimuli, a brief period of tonic hyperpolarization reduces relative firing rates over the stimulus window and is reliant on appropriate ionic concentrations in the extracellular matrix.[64–66] However, this ionic imbalance hypothesis cannot account for the absence of change in the response profile in TBI animals.

In the long-term postsurgery, TBI broadens the response profile in all cortical layers and P4 acts to narrow the response profile in the injured brain only.

We first consider the long-term effects on response profiles in TBI without P4 treatment. We now show that the previously demonstrated hyperactivity in supragranular layers at 8 wk post-TBI,[24,27] with normal input layer responses, is accompanied by broader response profiles in all cortical layers. The broadening in input layers implicates a broader thalamic input, possibly due to long-term downregulation of GABA activity[61] or as a function of imbalances in local cortical circuitry.[26] The complex stimuli presented here most likely engage networks in L2 which codes for stimulus feature components.[63] These layers show a loss of calretinin-labeled inhibitory neurons in the long term,[16] and this may lead to a broadening of response profiles in the TBI brain and a lack of sensory sensitivity.

At 8 wk post-TBI, the PFR increases only in supragranular layers,[24,27] whereas the temporal profiles broadened throughout the cortical column. These results show a clear disconnect between these 2 metrics of neuronal responses. We have previously argued that, in TBI, the supragranular layer-specific long-term hyperactivity indicates a TBI-induced dysfunction of the local inhibitory/excitatory balance.[26,27] This may be further confounded by the long-term establishment of inappropriate synaptic connections[15] and supragranular loss of dendrite-targeting calretnin-expressing inhibitory interneurons.[16] These physiological changes could account for a response broadening in upper cortical layers but not the changes seen throughout the cortical column. Interneurons types vary in molecular, morphological, and functional properties[67] with inhibitory neuron subclasses acting in functionally independent ways to modulate response properties[59] making it difficult to draw conclusions about dysfunction of a specific cell type in this research. We propose that the cross-laminar broadening of response profiles in long-term TBI may be a combination of cortical effects and downregulation of thalamic GABA activity[61] resulting in a temporally broader afferent signal to cortex.

With respect to P4 effects in long-term TBI, we first note our results that P4 had long-lasting effects in the sham brain[24] and caused long-term sharpening of response profiles in the TBI brain (present study), indicating that our dosing regimen was capable of eliciting effects into the long term in both sham and TBI brains. P4 sharpened temporal response profiles in TBI animals in U3 to L5 in the long term but had no systematic effect in sham animals. It is possible that the response profile sharpening in P4-treated TBI animals is a long-term consequence of short-term P4 actions that subsequently affect only the response profile but not response strength. Following TBI, P4 removes free radicals and decreases edema, limits lipid peroxidation and consequently curbs the size of BBB lesions,[57] regulates the expression of proinflammatory cytokines,[68] promotes vascular repair via upregulation of endothelial progenitor cells,[69] and limits mitochondrial dysfunction, stemming apoptosis and reducing excitatory amino acid induced excitotoxicity.[18,33,47,48,70–72] Amelioration of some of these short-term TBI-induced

pathologies by P4 may help to normalize some of the later pathological mechanisms responsible for the broad response profile seen across the entire cortical column in P4-untreated TBI animals. The sharpening of TBI responses in P4-treated animals to levels similar to those of sham animals may have a beneficial effect on sensory coding, specifically sensory discrimination, in long-term TBI.

Given our dosing regimen appears capable of causing long-term effects, the absence of long-term P4 effects in sham animals could be due to downregulation of cortical GABA receptors in the normal brain following long-term P4 exposure.[73,74] This adaptation to P4 does not appear to have occurred in the TBI cohort and it is tempting to link this to the loss of thalamic GABA activity in TBI[61] removing the substrate for adaptation of chronic P4 effects in TBI animals. Thus, the absence of P4 effects on the temporal response profile only in L2 of TBI animals may be due to inhibitory cell death and inappropriate synaptic connections in this layer.[15,16]

Conclusion

Our previous study showed no benefit of P4 treatment on the firing strength in the barrel cortex in either short-term or long-term post-TBI[24] and was consistent with clinical trials using P4 as a therapeutic option.[23] Here we show that P4 sharpens the response profile of whisker-driven cortical activity that is broadened by TBI, suggesting that P4 may provide benefits to sensory perception. It is impossible at this stage to know whether the sharpening of cortical firing profiles can be considered beneficial. However, the fact that we show P4 is having a long-term effect, maintaining the resolution of one component of sensory-based coding, is encouraging. Although P4 provided no benefit to motor function skills in our previous research, we had shown that P4 was providing marginal relief of sensory-based anxiety in the long term,[24] so it is not too far a bow to draw, to assume that the coding outcomes we show here are beneficial.

Due to the wealth of previous encouraging preclinical data concerning P4 treatment of TBI, including this study here, we agree with others[1] that a recalibration of P4 dosing, a better understanding of P4 action, and the optimal therapeutic window may lead to it being a viable treatment option of TBI. It may also be important to identify potential therapeutic options that may be combined with P4 for best clinical outcomes. For example, stem cells have been shown to differentiate into glia and neurons and attenuate motor deficits.[75] Injection of stem cells into the hippocampus following TBI has also shown to attenuate cognitive impairment and leads to the expression of glial-cell-line–derived neurotrophic factor.[76] Potential combinations of area-specific therapies at different time points post-injury could combine for positive clinical outcomes.

Authors' Note

The National Health and Medical Research Council did not contribute to study design, data collection, analysis, and manuscript preparation.

Ethical Approval

The protocols in this study were approved by the relevant ethics committee (see Materials and Methods).

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

Declaration of Conflicting Interests

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

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