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Whisker Stimulation increases expression of Nerve Growth Factor- and Interleukin-1 β -immunoreactivity in the Rat Somatosensory Cortex

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Abstract

Activity-dependent changes in cortical protein expression may mediate long-term physiological processes such as sleep and neural connectivity. In this study we determined the number of nerve growth factor (NGF)- and interleukin-1 β (IL1 β)-immunoreactive (IR) cells in the somatosensory cortex (Sctx) in response to 2 h of mystacial whisker stimulation. Manual whisker stimulation for 2 h increased the number of NGF-IR cells within layers II–V in activated Sctx columns, identified by enhanced Fos-IR. IL1 β -IR neurons increased within layers II–III and V–VI in these activated columns and IL1 β -IR astrocytes increased in layers I, II–III and V as well as the external capsule beneath the activated columns. These whisker-stimulated increases in the Sctx did not occur in the auditory cortex. These data demonstrate that expression of NGF or IL1 β in Sctx neurons and IL1 β in Sctx astrocytes is, in part, afferent input-dependent.

Keywords

use-dependent; astrocytes; cytokines; sleep; glial-neuronal interactions

1. Introduction

Neurotrophic growth factors and other cytokines are posited to play a role in activity-dependent cellular mechanisms (Levi-Montalcini, 1966; Cellerino and Maffei, 1996; Boulanger and Poo, 1999; Thoenen, 2000; Krueger et al., 2008). Nerve growth factor (NGF) is part of a larger neurotrophic family that includes brain-derived neurotrophic factor (BDNF), neurotrophic factor 3 (NT3) and neurotrophic factor 4 (NT4); most of which show activity-dependent expression (Ickes et al., 2000; Lu, 2004; Tabuchi, 2008). For instance, NGF mRNA levels upregulate in response to seizure activity (Gall and Isackson, 1989; Zafra et al., 1990; Ernfors

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et al., 1991), depolarizing stimuli (Lindholm et al., 1994; Lu, 2004) and afferent input from whiskers (Schwartz et al., 1994). NGF protein is released in response to neural activity (Blochl and Thoenen, 1995, 1996). Sleep deprivation, a method associated with enhanced neural activity (Vyazovskiy et al., 2009), increases the number of NGF-immunoreactive (IR) cells in the primary somatosensory cortex (Sctx) (Brandt et al., 2001). Neuronal expression of interleukin-1 beta (IL1 β) and tumor necrosis factor alpha (TNF), also are activity-dependent. For example, whisker stimulation increases the number of TNF-IR cells in Sctx neurons (Churchill et al., 2008). IL1 β mRNA levels are up-regulated by epileptiform activity in the hippocampus and cerebral cortex (Eriksson et al., 2000; Ravizza et al., 2006). Manipulations that mimic neuronal activity, such as depolarization, dramatically increase the levels of IL1 β released in hippocampal samples (Tringali et al., 1999). Induction of hippocampal long-term-potential (LTP) increases IL1 β mRNA levels (Schneider et al., 1998). Enhanced neuronal activation, as evidenced by Fos-IR in the reticular thalamus, is accompanied by an increase in IL1 β -IR in this region after cortical IL1 β application (Yasuda et al., 2007). Blocking olfactory input by closing the nares decreases levels of IL1 β -IR in the periglomerular region of the olfactory bulb (Lim and Brunjes, 1999).

Our interest in the neurotrophins/cytokines derives from their involvement in sleep regulation. All of the neurotrophins/cytokines mentioned have the capacity to enhance non-rapid eye movement (NREM) sleep and there is substantial evidence that TNF, IL1 β and NGF are involved in physiological sleep regulation (reviewed Krueger et al., 2008). Further, several modern theories of sleep mechanisms and function emphasize that sleep is dependent upon prior neuronal activity and is a fundamental property of highly interconnected neuronal networks such as cortical columns (Krueger and Obal, 1993; Kavanau, 1994; Benington and Heller, 1995; Tononi and Cirelli, 2003; Krueger et al., 2008). One test of these theories is to determine whether expressions of sleep regulatory substances such as NGF and IL1 β change in the Sctx under conditions that affect sleep. Electroencephalographic (EEG) slow wave power (SWA) during NREM sleep, a measure of sleep intensity, is altered by whisker stimulation (Vyazovskiy et al., 2000) or time of day (Yasuda et al., 2005a; Alfoldi et al., 1991). Thus, demonstration of the use-dependent expression of somnogenic neurotrophins and cytokines would be consistent with the proposal that sleep is a use-dependent property of localized networks.

The glial network has also been implicated as a candidate for mediating sleep homeostasis (Krueger et al 2008; Halassa et al., 2009). Whisker stimulation increases astrocytic cytosolic calcium in the Sctx (Wang et al., 2006; Schipke et al., 2008). Similarly, in the visual cortex (Vctx), astrocytic calcium levels respond to specific visual stimuli and these features are mapped in close register to the neuronal maps (Schummers et al., 2008). Astrocytes, like neurons, release ATP in response to cell activity (Pascual et al., 2005; Jourdain et al., 2007; Burnstock, 2007, 2009). ATP is posited to be the activity-indexed signal that initiates the process by which the brain keeps track of past activity (Krueger et al 2008). The purine type 2 receptors that respond to ATP release, in turn, are involved in processing pro-IL1 and subsequent release of mature IL1 β from astrocytes (Ferrari et al., 2006; reviewed Dinarello, 2009). The IL1 thus released is longer-lived than extracellular ATP and is involved in initiating sleepiness and sleep (reviewed Krueger et al 2008). Therefore we investigated whether IL1 β -IR astrocytes respond to whisker stimulation.

In the current study, we used a whisker stimulation model that is a well-characterized animal model for activity-induced gene expression (Mack and Mack, 1992; Melzer and Steiner, 1997; Filipkowski et al., 2000). For instance, unilateral stimulation of whiskers activates Fos expression in the corresponding Sctx columns on the opposite hemisphere (Melzer and Steiner, 1997; Filipkowski et al., 2000). We manually stimulated whiskers unilaterally for 2 h, then determined NGF- and IL1 β -IR in the Sctx. We found that stimulation increased the number of

NGF- and IL1 β -positive neurons and IL1 β -IR astrocytes and that NGF and IL1 β often co-localized in Sctx neurons.

2. Results

2.1 Whisker stimulation increased the number of NGF-IR cells in the activated columns of the Sctx as evidenced by the activity marker Fos

Fos-IR increased in a columnar pattern in the Sctx contralateral to the whisker stimulation (Fig. 2A-activated column is marked by the bar). The increased number of Fos-IR cells in the stimulated column was evident in all of the Sctx layers (Table 1). The number of NGF-IR cells (Fig. 2B) increased in layers II–V in an adjacent section. In the activated columns, the number of NGF-IR cells increased significantly in layers II–V but not in layer VI compared to the non-activated column (Table 1). No significant differences in NGF- or Fos-IR cells were observed between the stimulated and unstimulated sides in the auditory cortex.

2.2 Whisker stimulation increased the number of IL1 β -IR cells in the Sctx

The number of IL1 β -IR cells increased in the stimulated columns (Fig. 2C) as evidenced by the larger number of Fos-IR cells in these columns in the adjacent section (comparing Fig. 2A with 2C from the same rat). A significant increase in the number of IL1 β -IR pyramidal-shaped neurons occurred in layers II–III, V and VI in the stimulated columns compared to the adjacent unstimulated columns (Table 1). No significant differences in IL1 β -IR cells were observed between the stimulated and unstimulated sides in the auditory cortex. Additionally, the number of IL1 β -IR astrocytes in layers I, II–III and V as well as the underlying external capsule demonstrated significant increases in the stimulated columns (Table 2). However, no differences were observed between the two sides of the auditory cortex and its adjacent external capsule.

2.3 Morphology of NGF and IL1 β -IR cells

NGF-IR cells in layers II–VI appeared to be neurons based on the size and the pyramidal morphology of cell bodies with apical dendrites (Fig. 3). Whisker stimulation increased the density and number of NGF-IR neurons in the stimulated columns (Fig. 3B, D, E & H). IL1 β -IR cells in layers II–VI also appeared to be pyramidal-shaped neurons with apical dendrites or astrocytes (Fig. 4). The IL1 β -IR in neurons appeared in the cytoplasm surrounding the nucleus and extended into the apical dendrites. Whisker stimulation increased the density and number of IL1 β -IR neurons in the stimulated columns (Fig. 4B, D, E & H). Additionally, IL1 β -IR astrocytes in the external capsule increased in density in the stimulated column relative to the unstimulated column (Fig. 5).

2.4. Colocalization of IL1 β -IR with NeuN-, Fos- and NGF-IR cells

Double labeling immunohistochemistry demonstrated that IL1 β - pyramidal-shaped cells colocalized with the neuronal nuclear marker, NeuN (Fig. 6A). The nucleus was brightly stained by NeuN. Also IL1 β -IR stained the cytoplasm of cells containing Fos-IR in the nucleus (Fig. 6B). Also IL1 β -IR cells colocalized with NGF-IR cells (Fig. 6C).

3. Discussion

3.1 Activity dependent increases in NGF and IL1 β -IR cells

The major finding reported here is that whisker stimulation enhances the number of NGF and IL1 β -IR neurons in the Sctx. However, these changes were not observed in the auditory cortex, since each side of the cortex receives sensory inputs from both ears (Hutson et al., 2008). Previously it was shown that epileptic activity increases expression of NGF (Gall and Isackson,

1989; Zafra et al., 1990; Ernfors et al., 1991) and IL1 β (Eriksson et al., 2000; Ravizza et al., 2006) and whisker stimulation increases NGF mRNA levels (Schwartz et al., 1994) and the number of TNF-IR cells in the Sctx (Churchill et al., 2008). Further, natural whisker stimulation during sleep deprivation increases the number of NGF-IR cells in the Sctx, while a unilateral whisker trim decreases the number of these cells in the Sctx connected to this side (Brandt et al., 2001). These prior findings as well as the data reported herein, support the conclusion that localized neural activity regulates the level of SRSs.

In another group of rats that were killed at the same time as these rats (11 h after light onset) but without experimenter-induced whisker stimulation, the levels of Fos- and IL1 β -IR cells were similar to that observed in this study (Hight et al., in review). In those rats, the number of Fos- and IL1 β -IR cells were significantly greater when the rats were killed at 11 h after dark onset compared to when they were killed at the end of the light period, suggesting that the natural stimulation of the whiskers that occurs in the dark also enhances the levels of Fos and IL1 β -IR cells.

A second finding, that sensory stimulation enhances the number of IL1 β -IR astrocytes in the Sctx and the white matter layer below the Sctx, supports the hypotheses that astrocytes respond to neural activity and produce SRSs in response to neural activity. Whisker stimulation increases the calcium signaling in astrocytes within the Sctx (Wang et al., 2006) and visual stimulation increases calcium responses in specific columns of the Vctx (Schummers et al., 2008). In the current study, the increased number of IL1 β -IR astrocytes in the Sctx layers and the external capsule below the stimulated Sctx suggests that enhanced neural activity results in an increased expression of the cytokine IL1 β in astrocytes. These results are consistent with previous findings that the homeostatic response regulating ocular dominance in the visual cortex is mediated by astrocytic TNF (Kaneko et al., 2008) and together confirm the importance of astrocyte-neuron communication in neuronal networks (Fellin, 2009).

3.2 Colocalization of activity dependent increases in NGF and IL1 β

Our findings that IL1 β -IR colocalizes with NGF-IR within cells in the Sctx layer V is consistent with the previous findings that NGF and IL1 β colocalize in the frog nervous system during development (Jelaso and DeLong, 2005). The colocalization of NGF and IL1 β in Sctx neurons may be due to their mutual dependence on nuclear factor kappa B (NF- κ B); both are upregulated after NF- κ B activation (Carter et al., 1996; Cao et al., 1999; Mamidipudi et al., 2002; Mémet, 2006; Krueger et al., 2007). Further, an NGF-dependent association of interleukin 1 receptor associated kinase with the p75 neurotrophin receptor could explain, in part, the interaction between IL1 β and NGF in neurons and in immune cells (Mamidipudi et al., 2002; Friedman, 2005). Sleep deprivation-dependent increases in NF- κ B translocation into the nucleus, the main step in initiating transcription of a number of sleep-regulatory genes, occur in the cortex (Chen et al., 1999) and hypothalamus (Brandt et al., 2004). These observations suggest that cortical activity increases the nuclear translocation of NF- κ B and subsequent transcription of NGF and IL1 β mRNAs in an activity-dependent manner.

3.3 Network circuitry transferring the activity dependent increases in IL1 β and NGF to sleep regulatory centers

After application of IL1 β or TNF onto the somatosensory cortex, Fos- and IL1 β -IR neurons are induced in the somatic region of the reticular nucleus of the thalamus. Additionally, IL1 β -IR astrocytes increase in the perireticular zone of the internal capsule (Churchill et al., 2005; Yasuda et al., 2007). Furthermore, cortical application of IL1 β up-regulates Fos-IR cells in hypothalamic regions implicated in sleep regulation, such as the ventrolateral preoptic area and the median preoptic nucleus (Yasuda et al., 2007). These results as well as the increases in IL1 β -IR astrocytes in the external capsule support the idea that the local state initiated in

cortical columns by activity-induced SRSs is communicated via cortical connections to sleep regulatory circuits to coordinate whole organism sleep.

Previous studies in our lab suggest that unilateral cortical application of SRSs, such as IL1 β and TNF, increase sleep intensity evidenced by enhanced EEG SWA on the ipsilateral side (Yoshida et al., 2004; Yasuda et al., 2005b). Thus the whisker stimulation-induced IL1 β and NGF may provide an endogenous local source of SRSs that promote sleep within the stimulated cortical columns. Indeed, when rats are sleep deprived after unilateral whisker removal, the side ipsilateral to the cut whiskers receive disproportionate sensory stimulation and exhibit enhanced NGR-IR (Brandt et al 2001) and enhanced EEG delta power in subsequent sleep (Vyazovskiy et al., 2000; 2002). Multiple additional studies using measurements of EEG, or blood flow, or evoked response potentials, indicate that excessive afferent input to a region of the cortex is associated with deeper subsequent sleep (reviewed Krueger et al 2008). For instance, excessive right hand stimulation during wakefulness increases EEG SWA during subsequent sleep in the left cortex in humans (Kattler et al., 1994). Since rats increase their tactile and whisker activity and decrease their visual input during the dark, Sctx EEG SWA is higher during this time than that of the Vctx (Yasuda et al., 2005a). Conversely in the light, EEG SWA of the Vctx is higher than that of the Sctx. Further the probability of a cortical column entering a sleep-like state, as determined from higher amplitude evoked response potentials, is afferent activity-dependent (Rector et al., 2009).

3.4 Conclusion

In conclusion, the present study provides direct evidence that two SRSs, NGF and IL1 β , are up-regulated in the Sctx by excessive afferent input. Our findings add new evidence to the hypotheses that sleep is an activity-dependent event regulated in part by astrocytic and neuronal SRSs in the Sctx.

4. Experimental Procedures

4.1 Animals

Male Sprague-Dawley rats weighing 250–400 g were obtained from Taconic Farm, Inc. (Germantown, NY). Rats were housed on a 12 h/12 h light/dark cycle at 24 $\pm$ 2 $^{\circ}$ C. Seven rats were adapted to unilateral whisker stimulation as previously described (Four of these rats were the same rats analyzed in Group B in Churchill et al., 2008). Water and food were available ad libitum throughout the experiment. The use of rats was in accordance with Washington State University and international guidelines and was approved by the Institution of Animal Care and Use Committee.

4.2 Unilateral Whisker stimulation in a plexiform chamber

Rats were adapted to unilateral manual whisker stimulation outside of their home cages in a cage (40 cm by 40 cm) divided into two halves by plastic netting as previously described (Churchill et al., 2008). Briefly, the rats were adapted to the whicker stimulation over the course of 8 days starting with 15 min of stimulation and adding 15 min of stimulation time for each subsequent day. On the final day, the rats were stimulated for 2 h prior to the kill. Since the stimulation of Fos-IR in the barrel cortex after whisker stimulation was maximal after 1h and back to normal by 14 h (Bisler et al., 2002), we did not include a group that was adapted to whisker stimulation on all 8 days, but not stimulated on the day of the kill. Therefore, we didn't think that the adaptation days would have a long-term impact on IL1 or NGF-IR. Each investigator was assigned two rats, one for each hand. Each investigator followed each rat about the enclosed area brushing by hand the longer, more caudal mystacial whiskers (B0, B1, CO, C1, D0, D1, E1) on one side only at a rate of about 30 times per minute. The rats were killed at 10 min intervals starting at 1 h prior to dark onset. Following 2 h of unilateral whisker

stimulation, the rats were anesthetized with isoflurane and cardiac-perfused with warm saline followed by cold 4% paraformaldehyde. Brains were then post-fixed in this fixative for 2 h and sunk in 20% sucrose overnight. The brains were frozen in crushed dry ice and stored at -80°C until sectioning.

4.3 Immunohistochemistry

Brains were sectioned coronally with a sliding microtome at 30 μm thickness and collected at 360 μm intervals for each antigen. Immunohistochemistry was performed as described previously (Churchill et al., 2005, 2008), using a rabbit polyclonal anti-human Fos antibody (1:10,000; Calbiochem, Oncogene Research Products, San Diego, CA), goat anti-rat IL1 β (0.5 $\mu\text{g}/\text{ml}$, R&D Systems, Inc., Minneapolis, MN), rabbit anti-mouse NGF that cross reacts with rat NGF (1:2500 or 1:5000; Chemicon, Temecula, CA). These sections were incubated for 3 days at 4°C with gentle rocking.

Negative controls for the secondary antibodies were performed by omitting the primary antibody. The specificity of the NGF and Fos antibodies were previously published (Zhang et al., 2007; Berghorn et al., 1994). The specificity of the IL1 β antibody was tested by preincubation with an excess of IL1 β protein for 24 h at 4°C prior to the immunohistochemistry with the coronal sections. No specific IR-labeling was observed in the sections incubated without primary antibodies or with the preincubated IL1 β antibody (Fig. 1A) as compared with the specific rat Sctx labeling (Fig. 1B). This R & D antibody to rat IL1 β failed to show specific IR staining in IL1 β knockout mice (Fig. 1C relative to wild-type mice Fig. 1D) generously provided by Dr. M.K. O'Banion. Western blot analyses (Churchill et al., 2008) of extracts of lipopolysaccharide-(LPS) activated macrophages showed labeling of the 34 kD and 17kD forms of IL1 β by the IL1 β antibody; if cells were pretreated with IL1 β siRNA, labeling was reduced (Fig. 1E).

4.4 Imaging and Quantitative analysis

The Sctx and the auditory cortex (as a control cortical region) were identified in the brain sections as demarcated in the rat brain atlas by Paxinos and Watson (2007). The digital photographs were prepared on a Leica DMLB microscope with a Spot camera for quantitative analysis. The digital images were adjusted using Adobe Photoshop, only in contrast and brightness. A rectangular box was prepared using a transparent micron ruler for calibration and superimposed over each Sctx layer in the digital images. NGF-stained sections were photographed with the 10 $\times$ objective and two 0.5 mm $\times$ 0.2 mm boxes were superimposed onto each of the Sctx or auditory cortex layers. IL1 β -stained sections were photographed with a 20 $\times$ objective and one 0.5 mm $\times$ 0.225 mm box was superimposed onto each of the neuronal layers or three 0.23 mm by 0.08 mm boxes were superimposed onto the white matter layers. Fos-IR in an adjacent section was determined and used to identify stimulated columns on the side contralateral to the manual whisker stimulation. A stimulated column was identified where the number of Fos-IR cells in layer IV increased more than 25% compared with the adjacent columns. Columns of stimulated Fos expression were also observed on the ipsilateral side due to inadvertent whisker stimulation on the opposite side of the face; these columns were not used in the data analyses. The numbers of NGF-, IL1 β - and Fos-IR cells were counted for each layer in the stimulated and unstimulated columns as well as on both sides of the auditory cortex.

4.5 Statistical Analyses

A paired Student's *t*-test was used to evaluate the differences in the number of IR cells between the stimulated (Fos-IR evident) and unstimulated columns (little Fos-IR) on the stimulated side as well as between the stimulated and unstimulated sides of the auditory cortex.

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List of abbreviations

ATP	adenosine triphosphate
BDNF	brain-derived neurotrophic factor
EEG	encephalographic
GAPDH	glyceraldehyde-3-phosphate-dehydrogenase
IR	immunoreactivity, immunoreactive
IL1 β	interleukin-1 beta
LPS	lipopolysaccharide
LTP	long-term-potential
NGF	nerve growth factor
NeuN	neuronal nuclear protein
NF- κ B	Nuclear factor kappa B
NT3 and NT4	neurotrophin 3 and 4
NREMS	Non rapid eye movement sleep
PBS	phosphate buffered saline
siRNA	short-interfering RNA
Sctx	somatosensory cortex
SRSs	Sleep regulatory substances
SWA	slow wave activity or power
TNF	tumor necrosis factor alpha
Vctx	visual cortex

References

- Alfödi P, Franken P, Tobler I, Borbély AA. Short light-dark cycles influence sleep stages and EEG power spectra in the rat. *Behav Brain Res* 1991;43:25–31.
- Benington JH, Heller HC. Restoration of brain energy metabolism as the function of sleep. *Prog Neurobiol* 1995;45:347–360. [PubMed: 7624482]
- Berghorn KA, Bonnett JH, Hoffman GE. cFos immunoreactivity is enhanced with biotin amplification. *J Histochem Cytochem* 1994;42:1635–1642. [PubMed: 7983364]
- Bloch A, Thoenen H. Characterization of nerve growth factor (NGF) release from hippocampal neurons: evidence for a constitutive and an unconventional sodium-dependent regulated pathway. *Eur J Neurosci* 1995;7:1220–1228. [PubMed: 7582095]
- Bloch A, Thoenen H. Localization of cellular storage compartments and sites of constitutive and activity-dependent release of nerve growth factor (NGF) in primary cultures of hippocampal neurons. *Mol Cell Neurosci* 1996;7:173–190. [PubMed: 8726102]
- Boulanger LM, Poo MM. Presynaptic depolarization facilitates neurotrophin-induced synaptic potentiation. *Nat Neurosci* 1999;2:346–351. [PubMed: 10204541]

- Brandt JA, Churchill L, Guan Z, Fang J, Chen L, Krueger JM. Sleep deprivation but not a whisker trim increases nerve growth factor within barrel cortical neurons. *Brain Res* 2001;898:105–112. [PubMed: 11292453]
- Brandt JA, Churchill L, Rehman A, Ellis G, Mémet S, Israël A, Krueger JM. Sleep deprivation increases the activation of nuclear factor kappa B in lateral hypothalamic cells. *Brain Res* 2004;1004:91–97. [PubMed: 15033423]
- Burnstock G. Physiology and pathophysiology of purinergic neurotransmission. *Physiol Rev* 2007;87:659–797. [PubMed: 17429044]
- Burnstock G. Purinergic signaling: past, present and future. *Braz J Med & Biol Res* 2009;42:333–392.
- Cao Z, Tanaka M, Regnier C, Rothe M, Yamit-hezi A, Woronicz JD, Fuentes ME, Durnin MH, Dalrymple SA, Goeddel DV. NF-kappa B activation by tumor necrosis factor and interleukin-1. *Cold Spring Harb Symp Quant Biol* 1999;64:473–483. [PubMed: 11232324]
- Carter BD, Kaltschmidt C, Kaltschmidt B, Offenhäuser N, Böhm-Matthaei R, Baeuerle PA, Barde YA. Selective activation of NF-kappa B by nerve growth factor through the neurotrophin receptor p75. *Science* 1996;272:542–545. [PubMed: 8614802]
- Cellerino A, Maffei L. The action of neurotrophins in the development and plasticity of the visual cortex. *Prog Neurobiol* 1966;49:53–71. [PubMed: 8817698]
- Chen Z, Gardi J, Kushikata T, Fang J, Krueger JM. Nuclear factor-kappaB-like activity increases in murine cerebral cortex after sleep deprivation. *Am J Physiol* 1999;276:R1812–1818. [PubMed: 10362764]
- Churchill L, Yasuda K, Yasuda T, Blindheim KA, Falter M, Garcia-Garcia F, Krueger JM. Unilateral cortical application of tumor necrosis factor alpha induces asymmetry in Fos- and interleukin-1beta-immunoreactive cells within the corticothalamic projection. *Brain Res* 2005;1055:15–24. [PubMed: 16098952]
- Churchill L, Rector DM, Yasuda K, Fix C, Rojas MJ, Yasuda T, Krueger JM. Tumor necrosis factor: Activity dependent expression and promotion of cortical column sleep in rats. *Neuroscience* 2008;156:71–80. [PubMed: 18694809]
- Dinareello CA. Immunological and inflammatory function of the interleukin-1 family. *Annu Rev Immunol* 2009;27:519–550. [PubMed: 19302047]
- Eriksson C, Tehranian R, Iverfeldt K, Winflad B, Schultzberg M. Increased expression of mRNA encoding interleukin-1beta and caspase-1, and the secreted isoform of interleukin-1 receptor antagonist in the rat brain following systemic kainic acid administration. *J Neurosci Res* 2000;60:266–279. [PubMed: 10740232]
- Ernfors P, Bengzon J, Kokaia Z, Persson H, Lindvall O. Increased levels of messenger RNAs for neurotrophic factors in the brain during kindling epileptogenesis. *Neuron* 1991;7:165–176. [PubMed: 1829904]
- Fellin T. Communication between neurons and astrocytes: relevance to the modulation of synaptic and network activity. *J Neurochem* 2009;108:533–544. [PubMed: 19187090]
- Ferrari D, Pizzirani C, Adinolfi E, Lemoli RM, Curti A, Idzko M, Panther E, Di Virgilio F. The P2X7 receptor: a key player in IL-1 processing and release. *J Immunol* 2006;176:3877–3883. [PubMed: 16547218]
- Filipkowski RK, Rydz M, Berdel B, Morys J, Kaczmarek L. Tactile experience induces c-fos expression in rat barrel cortex. *Learn Mem* 2000;7:116–122. [PubMed: 10753978]
- Friedman WJ. Interactions of interleukin-1 with neurotrophic factors in the central nervous system: beneficial or detrimental? *Mol Neurobiol* 2005;32:133–144. [PubMed: 16215278]
- Gall CM, Isackson PJ. Limbic seizures increase neuronal production of messenger RNA for nerve growth factor. *Science* 1989;245:758–761. [PubMed: 2549634]
- Halassa MM, Florian C, Fellin T, Munoz JR, Lee SY, Abel T, Haydon PG, Frank MG. Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. *Neuron* 2009;61:213–219. [PubMed: 19186164]
- Hutson KA, Durham D, Imig T, Tucci DL. Consequences of unilateral hearing loss: cortical adjustment to unilateral deprivation. *Hear Res* 2008;237:19–31. [PubMed: 18261867]

- Ickes BR, Pham TM, Sanders LA, Albeck DS, Mohammed AH, Granholm AC. Long-term environmental enrichment leads to regional increases in neurotrophin levels in rat brain. *Exp Neurol* 2000;164:45–52. [PubMed: 10877914]
- Jelaso AM, DeLong C. NGF and IL-1beta are co-localized in the developing nervous system of the frog, *Xenopus laevis*. *Int J Dev Neurosci* 2005;23:575–586. [PubMed: 16143489]
- Jourdain P, Bergersen LH, Bhaukaurally K, Bezzi P, Santello M, Domercq M, Matute C, Tonello F, Gundersen V, Volterra A. Glutamate exocytosis from astrocytes controls synaptic strength. *Nat Neurosci* 2007;10:331–339. [PubMed: 17310248]
- Kaneko M, Stellwagen D, Malenka RC, Stryker MP. Tumor necrosis factor-alpha mediates one component of competitive, experience-dependent plasticity in developing visual cortex. *Neuron* 2008;58:673–680. [PubMed: 18549780]
- Kattler H, Dijk DJ, Borbély AA. Effect of unilateral somatosensory stimulation prior to sleep on the sleep EEG in humans. *J Sleep Res* 1994;3:159–164. [PubMed: 10607121]
- Kavanau JL. Sleep and dynamic stabilization of neural circuitry: a review and synthesis. *Behav Brain Res* 1994;63:111–126. [PubMed: 7999294]
- Krueger JM, Obal F Jr. A neuronal group theory of sleep function. *J Sleep Res* 1993;2:63–69. [PubMed: 10607073]
- Krueger JM, Rector DM, Churchill L. SLEEP AND CYTOKINES. *Sleep Med Clin* 2007;2:161–169. [PubMed: 19098992]
- Krueger JM, Rector DM, Roy S, Van Dongen HP, Belenky G, Panksepp J. Sleep as a fundamental property of neuronal assemblies. *Nat Rev Neurosci* 2008;9:910–919. [PubMed: 18985047]
- Levi-Montalcini R. The nerve growth factor: its mode of action on sensory and sympathetic nerve cells. *Harvey Lect* 1966;60:217–259. [PubMed: 5338067]
- Lim JH, Brunjes PC. Activity-dependent regulation of interleukin-1 beta immunoreactivity in the developing rat olfactory bulb. *Neuroscience* 1999;93:371–374. [PubMed: 10430500]
- Lindholm D, Castrén E, Berzaghi MP, Blochl A, Thoenen H. Activity-dependent and hormonal regulation of neurotrophin mRNA levels in the brain—implications for neuronal plasticity. *J Neurobiol* 1994;25:1362–1372. [PubMed: 7852991]
- Lu B. Acute and long-term synaptic modulation by neurotrophins. *Prog Brain Res* 2004;146:137–150. [PubMed: 14699962]
- Mack KJ, Mack PA. Induction of transcription factors in somatosensory cortex after tactile stimulation. *Mol Brain Res* 1992;12:141–147. [PubMed: 1312199]
- Mamidipudi V, Li X, Wooten MW. Identification of interleukin 1 receptor-associated kinase as a conserved component in the p75-neurotrophin receptor activation of nuclear factor-kappa B. *J Biol Chem* 2002;277:28010–28018. [PubMed: 12034707]
- Melzer P, Steiner H. Stimulus-dependent expression of immediate-early genes in rat somatosensory cortex. *J Comp Neurol* 1997;380:145–153. [PubMed: 9073089]
- Mémet S. NF-kappaB functions in the nervous system: from development to disease. *Biochem Pharmacol* 2006;72:1180–1195. [PubMed: 16997282]
- Pascual O, Casper KB, Kubera C, Zhang J, Revilla-Sanchez R, Sul JY, Takano H, Moss SJ, McCarthy K, Haydon PG. Astrocytic purinergic signaling coordinates synaptic networks. *Science* 2005;310:113–116. [PubMed: 16210541]
- Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. 6. San Diego, CA: Elsevier Academic Press; 2007.
- Ravizza T, Boer K, Redeker S, Spliet WG, van Rijen PC, Troost D, Vezzani A, Aronica E. The IL1beta system in epilepsy-associated malformations of cortical development. *Neurobiol Dis* 2006;24:128–143. [PubMed: 16860990]
- Rector DM, Schei JL, Van Dongen HP, Belenky G, Krueger JM. Physiological markers of local sleep. *Eur J Neurosci* 2009;29:1771–1778. [PubMed: 19473232]
- Schipke CG, Haas B, Kettenmann H. Astrocytes discriminate and selectively respond to the activity of a subpopulation of neurons within the barrel cortex. *Cereb Cortex* 2008;18:2450–2459. [PubMed: 18321876]

- Schneider H, Pitossi F, Balschun D, Wagner A, del Rey A, Besedovsky HO. A neuromodulatory role of interleukin-1beta in the hippocampus. *Proc Natl Acad Sci USA* 1998;95:7778–7783. [PubMed: 9636227]
- Schummers J, Yu H, Sur M. Tuned responses of astrocytes and their influence on hemodynamic signals in the visual cortex. *Science* 2008;320:1638–1643. [PubMed: 18566287]
- Schwartz RKW, Pei G, Söderström S, Ebendal T, Huston JP. Unilateral stimulation or removal of rat vibrissae: Analysis of nerve growth factor and tyrosine hydroxylase mRNA in the brain. *Behav Brain Res* 1994;60:63–71. [PubMed: 7910464]
- Tabuchi A. Synaptic plasticity-regulated gene expression: a key event in the long-lasting changes of neuronal function. *Biol Pharm Bull* 2008;31:327–335. [PubMed: 18310887]
- Thoenen H. Neurotrophins and activity-dependent plasticity. *Prog Brain Res* 2000;128:183–191. [PubMed: 11105678]
- Tononi G, Cirelli C. Sleep and synaptic homeostasis: a hypothesis. *Brain Res Bull* 2003;62:143–150. [PubMed: 14638388]
- Tringali G, Dello Russo C, Vairano M, Preziosi P, Navarra P. Depolarization and a NO-donor stimulate interleukin-1beta release from the rat hypothalamus via a mechanism involving caspase 1. *Neurosci Lett* 1999;276:119–122. [PubMed: 10624806]
- Vyazovskiy V, Borbély AA, Tobler I. Unilateral vibrissae stimulation during waking induces interhemispheric EEG asymmetry during subsequent sleep in the rat. *J Sleep Res* 2000;9:367–371. [PubMed: 11123523]
- Vyazovskiy V, Borbély AA, Tobler I. Interhemispheric sleep EEG asymmetry in the rat is enhanced by sleep deprivation. *J Neurophysiol* 2002;88:2280–2286. [PubMed: 12424269]
- Vyazovskiy VV, Olcese U, Lazimy YM, Faraguna U, Esser SK, Williams JC, Cirelli C, Tononi G. Cortical firing and sleep homeostasis. *Neuron* 2009;63:865–878. [PubMed: 19778514]
- Wang X, Lou N, Xu Q, Tian GF, Peng WG, Han X, Kang J, Takano T, Nedergaard M. Astrocytic Ca²⁺ signaling evoked by sensory stimulation in vivo. *Nat Neurosci* 2006;9:816–823. [PubMed: 16699507]
- Yasuda K, Churchill L, Yasuda T, Blindheim K, Falter M, Krueger JM. Unilateral cortical application of interleukin-1β (IL1β) induces asymmetry in fos, IL1β and nerve growth factor immunoreactivity: Implications for sleep regulation. *Brain Res* 2007;1131:44–59. [PubMed: 17184753]
- Yasuda T, Yasuda K, Brown RA, Krueger JM. State-dependent effects of light-dark cycle on somatosensory and visual cortex EEG in rats. *Am J Physiol Regul Integr Comp Physiol* 2005a;289:R1083–R1089. [PubMed: 16183627]
- Yasuda T, Yoshida H, Garcia-Garcia F, Kay D, Krueger JM. Interleukin-1β has a role in cerebral cortical state-dependent electroencephalographic slow-wave activity. *Sleep* 2005b;28:177–184. [PubMed: 16171241]
- Yoshida H, Peterfi Z, Garcia-Garcia F, Kirkpatrick R, Yasuda T, Krueger JM. State-specific asymmetries in EEG slow wave activity induced by local application of TNF alpha. *Brain Res* 2004;1009:129–136. [PubMed: 15120590]
- Zafra F, Hengerer B, Leibrock J, Thoenen H, Lindholm D. Activity-dependent regulation of BDNF and NGF mRNA in the rat hippocampus is mediated by non-NMDA glutamate receptors. *EMBO J* 1990;9:3545–3550. [PubMed: 2170117]
- Zhang HT, Li LY, Zou XL, Song XB, Hu YL, Feng ZT, Wang TT. Immunohistochemical distribution of NGF, BDNF, NT-3, and NT-4 in adult rhesus monkey brains. *J Histochem Cytochem* 2007;55:1–19. [PubMed: 16899765]

Fig. 1. Specificity of the antibody to rat recombinant IL1 β

A & B. Preabsorption of the IL1 β antibody with rat recombinant IL1 β blocks labeling of neuronal-like cells in layers II–IV that are observed after 2 h of whisker stimulation in B. C&D. Antibody to rat recombinant IL1 β does not label neuronal-like cells in the Sctx of the IL1 β knockout mice in comparison with the labeled cells in the Sctx of the wild type mouse in D. E. Lipopolysaccharide (LPS)-induced macrophages (cell line NR8383) treated with siRNA to IL1 β showed a reduction in the level of proIL1 β (34kD) and IL1 β (17kD) relative to the control (no siRNA-C) and the scrambled siRNA treatment (S). No differences were observed in the housekeeping protein-glyceraldehyde-3-phosphate-dehydrogenase (GAPDH).

Fig. 2. Fos-, NGF- and IL1 β -IR in the Sctx 2 h after whisker stimulation (Group A)

After 2 h of whisker stimulation, Fos-(A), NGF-(B) and IL1 β -(C) IR increased in a columnar pattern at similar positions in the Sctx contralateral to the whisker stimulation in adjacent sections. In the stimulated columns, NGF-, IL1 β - and Fos-IR cells were most prominent in layers II–III. The bar (0.7 mm) marks the stimulated columns. The unstimulated columns are observed on either side of the stimulated column.

Fig. 3. Cellular morphology of the NGF-IR cells

NGF-IR cells appear to be pyramidal shaped in layers II–V. NGF-IR appeared in the cytoplasm extending into the apical dendrites especially in layer V (H). Whisker stimulation increased the number of NGF-IR cells as well as the density of staining in the stimulated (stim) columns (B, D, F, H) when compared with the unstimulated (unstim) columns (A, C, E, G). Scale bars = 0.05 mm.

Fig. 4. Cellular morphology of the IL1 β -IR cells

In layers II–VI, the IL1 β -IR cells were pyramidal shaped. IL1 β -IR appeared in the cytoplasm extending into the apical dendrite in layer V (G & H). In layer I, the IL1 β -IR cells were shaped like astrocytes with a small nucleus and many branches (E & F). Whisker stimulation increased the number of IL1 β -IR cells and astrocytes in the stimulated (stim) columns (B, D, R, H) when compared with the unstimulated (unstim) columns (A, C, E, G). Scale bars = 0.05mm.

Fig. 5. IL1 β -IR astrocytes in the external capsule below the Sctx columns

IL1 β -IR appeared in the cytoplasm extending into the branches of the fibrous astrocytes in the external capsule. Whisker stimulation increased the number as well as the density of IL1 β -IR astrocytes (arrows) in the stimulated (stim) column (B) when compared with the unstimulated (unstim) column (arrow)(A). Scale bar=0.05 mm.

Fig. 6. IL1 β -IR cells colocalize with the neuronal nuclear marker, NeuN, Fos and NGF

A. IL1 β -IR cells (red) colocalized with the neuronal nuclear marker, NeuN (green) in layer V of the stimulated column in the barrel field (arrow). Scale bar = 20 μ m. **B.** IL1 β -IR (red) appeared in the cytoplasm of some Fos-IR (green) cells in the stimulated columns (arrow). Scale bar = 10 μ m. **C.** NGF-(green) and IL1 β (red)-IR colocalized in the cytoplasm of layer V Sctx neurons after 2 h of whisker stimulation (arrow). Scale bar = 20 μ m.

Table 1

Quantitative evaluation of the number of NGF- Fos-, and IL1 β -IR cells in stimulated versus unstimulated columns of the Sctx layers at 2 h after unilateral whisker stimulation. For comparison, the number of NGF-, Fos-, and IL1 β -IR cells in the Sctx layers in another group of unstimulated rats killed at the same time of day was illustrated. Data are represented as Mean $\pm$ S.E.M. for 7 rats. For the auditory cortex, NGF data are represented for 7 rats, while Fos and IL1 β data are represented by 6 rats.

Layers	Number of NGF-IR cells/0.1 mm ²				
	Sctx stim col in stim rat	Sctx unstim col in stim rat	prob	Aud ctx Stim side	Aud ctx Unstim
II-III	79.6 $\pm$ 18.1*	60.1 $\pm$ 6.1	p = 0.01	86.3 $\pm$ 10.8	87.9 $\pm$ 6.7
IV	57.7 $\pm$ 5.7*	46.3 $\pm$ 4.7	p = 0.01	60.6 $\pm$ 7.2	67.9 $\pm$ 7.7
V	48.0 $\pm$ 3.3*	38.3 $\pm$ 4.0	p = 0.001	51.4 $\pm$ 5.5	57.3 $\pm$ 4.3
VI	45.7 $\pm$ 4.8	41.1 $\pm$ 3.8	p = 0.10	66.4 $\pm$ 8.1	71.2 $\pm$ 6.0
Number of Fos-IR cells/0.1 mm ²					
II-III	128.1 $\pm$ 12.1*	83.7 $\pm$ 11.7	p = 0.012	108.0 $\pm$ 10.5	112.1 $\pm$ 12.3
IV	136.2 $\pm$ 14.5*	79.3 $\pm$ 14.8	p = 0.0004	106.7 $\pm$ 12.8	99.4 $\pm$ 12.1
V	91.2 $\pm$ 6.9*	73.4 $\pm$ 5.9	p = 0.0005	76.7 $\pm$ 8.8	82.3 $\pm$ 4.6
VI	96.1 $\pm$ 10.5*	80.3 $\pm$ 6.9	p = 0.012	108.8 $\pm$ 6.8	121.3 $\pm$ 5.8
Number of IL1 β -IR cells/0.1125 mm ²					
II-III	19.3 $\pm$ 12.3*	13.8 $\pm$ 3.4	p = 0.04	27.9 $\pm$ 4.4	31.3 $\pm$ 5.0
IV	11.2 $\pm$ 1.0	8.8 $\pm$ 1.9	p = 0.09	26.8 $\pm$ 5.7	26.1 $\pm$ 4.8
V	25.1 $\pm$ 2.2*	18.9 $\pm$ 2.4	p = 0.003	23.3 $\pm$ 2.6	21.5 $\pm$ 2.3
VI	14.6 $\pm$ 2.4*	12.0 $\pm$ 2.5	p = 0.04	28.0 $\pm$ 4.4	28.1 $\pm$ 4.0

Table 2

Number of IL1β-IR fibrous astrocytes/0.0552 mm ²					
	ScTx Layers			Auditory ctx Layers	
	Stim col	Unstim col		Stim side	Unstim side
I	14.7 ± 2.0*	8.8 ± 0.7	p = 0.007	11.4 ± 0.2	11.4 ± 0.5
Ext. capsule	23.3 ± 1.3*	16.0 ± 1.2	p = 0.004	11.9 ± 1.2	12.0 ± 1.2
Number of IL1β-IR protoplasmic astrocytes/0.1125 mm ²					
II-III	6.7 ± 0.5*	3.2 ± 0.8	p = 0.006	7.9 ± 0.7	7.2 ± 0.6
IV	1.9 ± 0.3	2.0 ± 0.3	p = 0.6	7.9 ± 0.7	7.5 ± 0.5
V	3.9 ± 0.5*	3.1 ± 0.5	p = 0.04	6.8 ± 0.7	7.0 ± 0.7
VI	3.9 ± 0.7	3.1 ± 0.6	p = 0.1	8.9 ± 1.5	8.8 ± 0.9

* p<0.05 comparing stimulated and unstimulated columns/sides using a paired Students t test