Properties of an Adult Spinal Sensorimotor Circuit Shaped Through Early Postnatal Experience

Per Petersson, Marcus Granmo, and Jens Schouenborg

Section for Neurophysiology, Department of Physiological Sciences, University of Lund, BMC F10, S-221 84 Lund, Sweden

Submitted 20 January 2004; accepted in final form 23 February 2004

INTRODUCTION

Sensorimotor circuits must incorporate detailed information on the biomechanics of the body to adequately perform rapid correction of movements. How this information is acquired and stored in different neuronal circuits is a fundamental question. We have recently shown that experience-dependent adjustments that functionally adapt the nociceptive withdrawal reflexes (NWRs) take place during development (Petersson et al. 2003; Waldenstrom et al. 2003). In adult mammals, the NWR system has a modular organization (Schouenborg 2002). Each module controls a single or a few synergistic muscles and transforms sensory input from a receptive field (RF) into appropriate motor activity. The sensitivity distribution within the cutaneous excitatory RF of a module mimics its withdrawal efficiency. In rats, this imprint of the withdrawal efficiency is engraved on the reflex pathways during the first three postnatal weeks (depending on body part) through extensive adjustments whereby erroneous connections are eliminated, or reduced, and the strength of adequate connections become proportional to withdrawal efficiency (Holmberg and Schouenborg 1996b; Waldenstrom et al. 2003). This process takes ~1 wk. Sensory deprivation experiments and behavioral studies, using artificial tactile feedback on spontaneous movements occurring during this time period, indicate that the imprint of withdrawal efficiency is formed by tactile feedback on spontaneous movements (Petersson et al. 2003; Waldenstrom et al. 2003). Computer simulation has shown that an unsupervised correlation-based learning mechanism, using tactile feedback from spontaneous muscle twitches, can account for the functional adaptation of the withdrawal reflex system (Petersson et al. 2003). This novel learning mechanism was termed “motor-directed somatosensory imprinting” (MDSI). Through MDSI, the sensorimotor transformation can adapt to neonatal alterations of both peripheral innervation (Holmberg and Schouenborg 1996a) and movement patterns (Holmberg et al. 1997).

Although a basic knowledge of the learning mechanisms that adapt spinal circuits to the body constitution during development has been obtained, it is not known which properties constitute the difference between strong and weak connections—that is, what properties constitute the end product of the postnatal learning. For example, it is not known if inhibitory connections or the types of excitatory postsynaptic receptors differ between strong and weak connections of the reflex circuit. As a step toward clarifying this matter, a comprehensive study of the functional properties of connections with different strengths within excitatory and inhibitory RFs of the NWR system was carried out. First the excitatory RFs of single reflex modules were characterized with respect to signal gain, response latency and variability. Second, we analyzed the inhibitory input to these reflex modules with respect to spatial organization, latency and strength. Third, the functional role of glutamate receptors [alpha-aminoo-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA/kainate) and N-methyl-D-aspartate (NMDA)] in the NWR sensorimotor transformation was studied in pharmacological experiments.

METHODS

Adult Wistar rats (n = 31) of both sexes were used. The animals received food and water ad libitum and were kept in a 12-h day-night cycle at a constant environmental temperature of 21°C (humidity: 65%). Approval for the experiments was obtained in advance from the Malmö/Lund ethical committee on animal experiments.

Address reprint requests and other correspondence to: P. Petersson (E-mail: Per.Petersson@mphy.lu.se).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Surgery and preparation

The animals were anesthetized with halothane (0.9%–2.0%), in a mixture of 65% nitrous oxide-35% oxygen, and were ventilated artificially via a tracheal cannula. The expiratory CO₂ (3.0–4.0%) was monitored continuously. An infusion of 5% glucose in Ringer acetate (pH = 7.0) at a rate of 40–80 μl/min was administered via the right jugular vein. Mean arterial blood pressure (65–140 mmHg) was monitored continuously in the right carotid artery. Core temperature was maintained between 36.5 and 38.5°C using a thermostatically controlled, feedback-regulated heating system. Local infiltration of 3.5 mg/ml lidocaine (Xylocaine) with 2.2 μg/ml adrenaline was used to reduce nociceptive input during surgery. A laminectomy of the 10th thoracic vertebrae was carried out, and the rat was spinalized using a pair of fine scissors. A craniotomy was performed, and the brain rostral to the inferior colliculus was removed. The anesthesia was then discontinued. In experiments used for pharmacological interventions, a laminectomy of the 13th thoracic, first and second lumbar vertebrae was made and the underlying meninges were removed. An agar pool with artificial cerebrospinal fluid (ACSF) (Edwards et al. 1989) over the lumbar cord was then created. Experiments were terminated on signs of deterioration, such as a precipitous drop in blood pressure or in expiratory CO₂ level. After termination of the experiments, the animals were given a lethal dose of halothane.

Cutaneous stimulation and electromyographic recordings

Nociceptive stimulation was performed either with CO₂ laser [beam diameter: 3 mm, intensity: 5 W, pulse duration: 12–28 ms (60–140 μJ)], resulting in an estimated maximal temperature of between 43 and 64°C at a depth of 100 μm (Haimi-Cohen et al. 1983) once every 1.5–2 min or by intracutaneous cathodal electrical stimulation using fine steel needle electrodes (n = 16, see following text). No visible damage of the skin, or marked changes in response properties at the stimulation sites, was detectable at these intensities.

The needles used for skin stimulation and electromyographic (EMG) recordings were electrolytically pointed and insulated with a varnish coating except for ~40 and 80 μm, respectively, at the tip. A small metal plate, used as an anode, was placed subcutaneously well outside the stimulation area. For EMG recordings, a small opening was made in the skin overlying the muscle, and the electrode was inserted into the mid-region of each muscle belly. A reference electrode was inserted in an adjacent skin flap. Generally, the EMG activity in two or three hind limb muscles was recorded simultaneously in each experiment.

RF mapping

A computerized method, termed RF imaging (RFI), for rapid mapping of multiple RFS and their respective sensitivity distributions was used in all experiments (Petersson et al. 2001). Automated stimulation and recording, with spike detection and counting, were performed on-line by this system. All raw data sweeps were stored to permit further off-line analyses. The RFI system allows repeated RF measurements in a time range of minutes. The RFS obtained with CO₂ laser and electrical stimulation were almost identical as has been demonstrated peristimulus histograms recorded at a certain stimulation intensity were summed. The accumulated peristimulus histogram was then smoothed by grouping the 2-ms time bins, five by five, into 10-ms time bins. The onset latency was defined as the time passed from stimulus onset until the first 10-ms bin containing at least half the number of spikes of the bin with the highest number—a method previously found to yield accurate estimates of onset latencies (Friedman and Priebe 1998). All histograms were also inspected visually and if responses were judged too small to allow reliable latency estimations these values were excluded from the automated process. A low spontaneous activity in all latency experiments enabled the use of this relatively simple algorithm, yielding estimates similar to the ones obtained by visual inspection of the spike arrival trains. Response latencies <100 ms (judged as A-fiber responses) and latencies >400 ms (activity likely arising from other sources) were not included. When compensating for differences in afferent fiber length a C-fiber conduction velocity of 0.8 m/s was assumed (Gee et al. 1996).

Pharmacological experiments

The exposed lumbar spinal cord was kept in a bath of ACSF (bath volume: ~300 μl). The response values for ≤16 sites were determined every 3 min before, during, and after the period with drug effect. The baseline was defined as at least five mappings with a fixed stimulation intensity yielding values with no trend to decrease or increase during a time-period of ~12 min. After the baseline measurement, the ACSF was removed by gentle suction with a syringe, then 6-nitro-7-sulfamoylbenzo(f)quinoxaline-2,3-dione (NBQX; 1–10
RESULTS

In the first set of experiments, we examined the properties of strong and weak connections in the NWR system. Differences with regard to gain, onset latency, and variability for different sites of the excitatory RFs were characterized. Skin sites of the hind paw were stimulated using a CO₂ laser (pulse duration ranging from 12 to 26 ms). RFs of NWRs of the peroneus longus muscle (PL, \( n = 17 \); pronator of the hind paw), the extensor digitorum longus muscle (EDL, \( n = 6 \); dorsiflexor of the digits and ankle), and the semitendinosus muscle (ST, \( n = 15 \); knee-flexor and adductor of hind limb) were mapped in 18 rats (Fig. 1, A–C).

Input-output relations

In previous studies, the RFs of hind-limb muscles have been mapped using constant stimulation intensity. To obtain a more detailed description, the relative reflex gain for individual skin sites within the RFs was mapped (\( n = 656 \) mappings) by applying CO₂ laser stimulation with different pulse duration. Thus the entire input-output relationship was determined for \( \leq 16 \) skin sites per RF. The EMG responses from three hind limb muscles, PL (\( n = 6 \) RFs), EDL (\( n = 4 \) RFs), and ST (\( n = 5 \) RFs), were mapped in seven animals. As can be seen in Fig. 2A, every site within each RF tended to respond with a certain fraction of the maximum response obtained in the field focus at each stimulation intensity. As a consequence, the intrinsic order of responsiveness of sites within the RFs was conserved regardless of the excitability changes that occurred during the experiments (Schouenborg et al. 1992). When gauging the responses from PL and EDL, with stimulations ranging from near-response nociceptive threshold to near-response saturation, the response amplitudes for sites within the RFs increased linearly with increasing stimulation intensity until a plateau was reached (linear regression; PL: \( r = 0.80 \); EDL: \( r = 0.82 \)).

Stimulus response latencies

To determine if there was a specific latency distribution within the RFs, we made 612 mappings (PL, \( n = 12 \) RFs; EDL, \( n = 4 \) RFs; and ST, \( n = 8 \) RFs) in 13 animals (laser pulse duration: 18–26 ms). The onset latency was measured for all sites, and maps of onset latency were constructed (see METHODS for details on latency measurements). In most experiments, individual onset latency for sites within the RFs decreased only slightly (mean maximum difference: \( \sim 50 \) ms in 8 experiments) with increasing stimulation intensity, whereas response amplitudes at the same time differed greatly (typically \( \geq 5 \) times; Fig. 3, C–E). The latency differences within the RFs had a distinct spatial distribution very much akin to the response amplitude distribution of the same muscle (Fig. 3, A and B; cf. Fig. 1A; mean maximum difference \( \sim 150 \) ms in 11 experiments).

In several neuronal systems, the distribution of inter-spike intervals (ISIs) of the first few spikes in each spike train approximately follow an exponential probability density so that the time to the next spike depends on the mean firing rate but is otherwise random (a Poisson process) (Shadlen and Newsome 1998). However, in the NWR system a Poisson process description seems not to be applicable as the onset latency is, in many cases, independent of stimulation intensity (in contrast to the response amplitude) but has clear dependence on site of stimulation. To verify this, ISIs of the first spikes in individual spike trains were assessed in three experiments to control for the possible role of differing initial firing rates on the measured onset latencies. It was confirmed that the differences in mean ISIs were typically \( < 10 \) ms—that is, considerably less than most measured latency differences between different sites. This finding supports the notion that latency differences are not due to different mean firing frequencies but rather are one of the specific features of different RFs, every site within each RF tended to respond with a certain fraction of the maximum response obtained in the field focus at each stimulation intensity. As can be seen in Fig. 2A, every site within each RF tended to respond with a certain fraction of the maximum response obtained in the field focus at each stimulation intensity. As a consequence, the intrinsic order of responsiveness of sites within the RFs was conserved regardless of the excitability changes that occurred during the experiments (Schouenborg et al. 1992). When gauging the responses from PL and EDL, with stimulations ranging from near-response nociceptive threshold to near-response saturation, the response amplitudes for sites within the RFs increased linearly with increasing stimulation intensity until a plateau was reached (linear regression; PL: \( r = 0.80 \); EDL: \( r = 0.82 \)).

Stimulus response latencies

To determine if there was a specific latency distribution within the RFs, we made 612 mappings (PL, \( n = 12 \) RFs; EDL, \( n = 4 \) RFs; and ST, \( n = 8 \) RFs) in 13 animals (laser pulse duration: 18–26 ms). The onset latency was measured for all sites, and maps of onset latency were constructed (see METHODS for details on latency measurements). In most experiments, individual onset latency for sites within the RFs decreased only slightly (mean maximum difference: \( \sim 50 \) ms in 8 experiments) with increasing stimulation intensity, whereas response amplitudes at the same time differed greatly (typically \( \geq 5 \) times; Fig. 3, C–E). The latency differences within the RFs had a distinct spatial distribution very much akin to the response amplitude distribution of the same muscle (Fig. 3, A and B; cf. Fig. 1A; mean maximum difference \( \sim 150 \) ms in 11 experiments).

In several neuronal systems, the distribution of inter-spike intervals (ISIs) of the first few spikes in each spike train approximately follow an exponential probability density so that the time to the next spike depends on the mean firing rate but is otherwise random (a Poisson process) (Shadlen and Newsome 1998). However, in the NWR system a Poisson process description seems not to be applicable as the onset latency is, in many cases, independent of stimulation intensity (in contrast to the response amplitude) but has clear dependence on site of stimulation. To verify this, ISIs of the first spikes in individual spike trains were assessed in three experiments to control for the possible role of differing initial firing rates on the measured onset latencies. It was confirmed that the differences in mean ISIs were typically \( < 10 \) ms—that is, considerably less than most measured latency differences between different sites. This finding supports the notion that latency differences are not due to different mean firing frequencies but rather are one of the specific features of

\[ \text{APs} \] or d-2-amino-5-phosphonovalerate (AP-5; 0.1–1 \( \mu \)g; Tocris) diluted in 100 \( \mu \) ACSF and titrated to pH 7.4 were administered into the bath. The effect of the drug stabilized 5–20 min after administration, and responses remained at a lower level plateau (a marked decrease but with reliable responses from most of the stimulation sites). At least six mappings during a time-period of \( \geq 15 \) min were made. The drug was then gradually washed out with ACSF (see Fig. 6). The doses of the glutamate antagonists used were within the range of effective doses found previously in similar models of spinal nociceptive processing (Bach and Yakhsh 1995; Jhamandas et al. 1996; Nishiyama et al. 1998; Zahn et al. 1998).

In the first set of experiments, we examined the properties of strong and weak connections in the NWR system. Differences with regard to gain, onset latency, and variability for different sites of the excitatory RFs were characterized. Skin sites of the hind paw were stimulated using a CO₂ laser (pulse duration ranging from 12 to 26 ms). RFs of NWRs of the peroneus longus muscle (PL, \( n = 17 \); pronator of the hind paw), the extensor digitorum longus muscle (EDL, \( n = 6 \); dorsiflexor of the digits and ankle), and the semitendinosus muscle (ST, \( n = 15 \); knee-flexor and adductor of hind limb) were mapped in 18 rats (Fig. 1, A–C).

Input-output relations

In previous studies, the RFs of hind-limb muscles have been mapped using constant stimulation intensity. To obtain a more detailed description, the relative reflex gain for individual skin sites within the RFs was mapped (\( n = 656 \) mappings) by applying CO₂ laser stimulation with different pulse duration. Thus the entire input-output relationship was determined for \( \leq 16 \) skin sites per RF. The EMG responses from three hind limb muscles, PL (\( n = 6 \) RFs), EDL (\( n = 4 \) RFs), and ST (\( n = 5 \) RFs), were mapped in seven animals. As can be seen in Fig. 2A, every site within each RF tended to respond with a certain fraction of the maximum response obtained in the field focus at each stimulation intensity. As a consequence, the intrinsic order of responsiveness of sites within the RFs was conserved regardless of the excitability changes that occurred during the experiments (Schouenborg et al. 1992). When gauging the responses from PL and EDL, with stimulations ranging from near-response nociceptive threshold to near-response saturation, the response amplitudes for sites within the RFs increased linearly with increasing stimulation intensity until a plateau was reached (linear regression; PL: \( r = 0.80 \); EDL: \( r = 0.82 \)).

Stimulus response latencies

To determine if there was a specific latency distribution within the RFs, we made 612 mappings (PL, \( n = 12 \) RFs; EDL, \( n = 4 \) RFs; and ST, \( n = 8 \) RFs) in 13 animals (laser pulse duration: 18–26 ms). The onset latency was measured for all sites, and maps of onset latency were constructed (see METHODS for details on latency measurements). In most experiments, individual onset latency for sites within the RFs decreased only slightly (mean maximum difference: \( \sim 50 \) ms in 8 experiments) with increasing stimulation intensity, whereas response amplitudes at the same time differed greatly (typically \( \geq 5 \) times; Fig. 3, C–E). The latency differences within the RFs had a distinct spatial distribution very much akin to the response amplitude distribution of the same muscle (Fig. 3, A and B; cf. Fig. 1A; mean maximum difference \( \sim 150 \) ms in 11 experiments).

In several neuronal systems, the distribution of inter-spike intervals (ISIs) of the first few spikes in each spike train approximately follow an exponential probability density so that the time to the next spike depends on the mean firing rate but is otherwise random (a Poisson process) (Shadlen and Newsome 1998). However, in the NWR system a Poisson process description seems not to be applicable as the onset latency is, in many cases, independent of stimulation intensity (in contrast to the response amplitude) but has clear dependence on site of stimulation. To verify this, ISIs of the first spikes in individual spike trains were assessed in three experiments to control for the possible role of differing initial firing rates on the measured onset latencies. It was confirmed that the differences in mean ISIs were typically \( < 10 \) ms—that is, considerably less than most measured latency differences between different sites. This finding supports the notion that latency differences are not due to different mean firing frequencies but rather are one of the specific features of...
Variance

The variance of the input signal for different sites could be expected to be scaled-up proportionally to the square of the gain in the circuit (noise amplification). If, however, different connections vary in properties other than the relative gain, these properties could be reflected in the relation between mean response amplitude and the variation of these responses. We therefore studied the variability in response amplitudes over multiple mappings using cutaneous CO₂ laser stimulation PL (n = 7 RFs), EDL (n = 3 RFs), and ST (n = 5 RFs) in eight animals. The variance increased monotonically with the mean response amplitude of the different sites. Approximate relationships can be formulated based on the median values of linear regressions in the log-log plots of all experiments as $\text{Var} \sim 13.3 \times (\text{response})^{0.91}$ for PL, $\text{Var} \sim 28.6 \times (\text{response})^{0.82}$ for EDL, and $\text{Var} \sim 10.8 \times (\text{response})^{0.93}$ for ST (PL and ST are shown in Fig. 4, A and B). An almost linear relationship was evident between variance and mean responses for different sites within the RF. The amount of variability within the RF could, therefore, not be directly explained by the difference in relative gain for different sites. Rather it appears that sites with higher relative gain somehow differ in signal transduction properties to sites with a lower gain.

When using natural stimulation, some noise will be introduced at the level of the peripheral receptors. This variance is independent of the central mechanisms and can thus be treated as a simple addition to the total variance. Because of the modular organization of the reflex for different muscles, the shared variance between two muscles provides a rough estimate of the peripheral variability and can therefore convey the connections from each site. Notably, the onset latency, in itself, carries the information about which site within the RF has been stimulated and could therefore potentially be used by other systems to quickly read out the cutaneous nociceptive input (c.f. Garwicz et al. 2002).

Organized overlying inhibitory input

Previous studies have shown that the inhibitory input from the skin is organized in an analogous way to that of the excitatory input such that the inhibition is related to the size of the movement component directed toward stimulation (Schouenborg and Weng 1994). Excitatory and inhibitory inputs have been shown to overlap to some extent (Schouenborg et al. 2001; Sonnenborg et al. 2000; Weng and Schouenborg 1996a). To clarify if overlapping inhibitory inputs contribute to the differences between strong and weak connections, the inhibitory nociceptive input was analyzed using pair wise stimulation. The control stimulation, producing excitation in the reflex pathway, was caused either by stimulation of the

FIG. 3. Onset latencies of the PL muscle. A: median time of onset latency above the shortest onset latency within the excitatory receptive field in each experiment (ms). B: the data in A are compensated for the delay in conduction time depending on spatial location (0.8 m/s conduction velocity assumed; mean SD of latencies for the same sites in different experiments = 48 ms). C and D: effects of varying stimulation intensity shown for a single experiment. C: response amplitudes increase with increasing stimulation intensity for the 6 sites shown, whereas in D, latency measurements in the same mappings show no clear relationship between latencies and stimulation intensity. E: location of the 6 sites within the PL receptive field in this experiment. Symbols in E correspond to those in C and D.
Excitatory fields. In alternative experiments, random stimulation within the excitatory RF. The results were similar
to those found previously (Weng and Schouenborg 1996a) and additionally showed that the inhibitory RF for each muscle was
independent of the site used to induce excitation (Fig. 5, A and B). The onset latency of inhibition varied within the inhibitory
RF in a manner similar to that of the excitatory RF, such that
the shortest latencies of inhibition were found for skin sites
producing maximal inhibition (data not shown). It is worth
noting that this means that their respective response latency
patterns cannot be explained by influences from the opposing
field. These findings indicate that there are distinct inhibitory
RFs in the NWR system in parallel to the excitatory RFs.

Effects of glutamate receptor antagonists

The RFI system used allowed for rapid reliable RF mapping
using electrical stimulation. In this arrangement, each site
was stimulated multiple times in each RF mapping (compared
with just 1 site with the laser), and mappings could be made
more often without the risk of receptor sensitization/desensitization.
Thus a high sensitivity to RF changes and a good temporal
resolution was reached. However, this was achieved at the cost
of using a more artificial type of stimulation. In the pharma-
ocological experiments, where a high sensitivity to RF dynamics
is advantageous to reliably track the effect of the applied drugs,
both natural and electrical stimulation (10 stimulations/site and
mapping, starting every 3rd minute) were used. CO2 laser
stimulation was used in seven rats’ PL (n = 6 RFs), EDL (n =
3 RFs), and ST (n = 3 RFs) and electrical stimulation in six
rats’ PL (n = 4 RFs), EDL (n = 3 RFs), and ST (n = 4 RFs).
Hypothetically, differences in amplitude, latency, and variation
within the excitatory RF could be due to differences in glutamate
receptors in the reflex pathways from different skin sites.
It has been suggested that the AMPA/kainate to NMDA recep-
tor ratio (A/N) differs between strong and weak synapses
(Takahashi et al. 2003). Thus if the non-NMDA-to-NMDA
receptor ratio differs for different connection strengths, the
relative effects of selective antagonists would be expected
to vary. To assess this possibility, we investigated the effects
of NBQX (n = 205 mappings in nine experiments) and AP-5
(n = 202 mappings in 10 experiments) for three different
muscles. The drugs were applied topically onto the spinal cord.
Both antagonists caused a response decrease, but the degree to
which strong and weak connections were affected differed.
AP-5 caused a response decrease almost exactly proportional
to the strength of the connections for all sites (Fig. 7, A and B),
whereas NBQX application resulted in an absolute decrease of
response regardless of site (weaker sites stopped responding)
and only a moderate proportional decrease (Fig. 7, C and D).
This was found in experiments using laser and electrical stimu-
lation. A similar effect of the antagonists on gain/threshold
was also found for individual sites in experiments evaluating
input-output relations (pulse duration 16–26 ms; n = 2 exper-
mements; data not shown). These results are at variance with the
view that there are major differences between strong and weak
connections with regard to receptor populations (see APPENDIX
for further details).

Discussion

As mentioned in the introduction, the sensitivity distributions
within the RFs of withdrawal reflex modules emerge
during development as a consequence of learning and consti-
tute “imprints” of the withdrawal movement patterns of
the output muscles. The present study provides a comprehensive
description of this imprint and thus information about the end
product of the learning process. In particular, differences be-
tween connections of different strengths with respect to gain,
lateness, variance, inhibitory inputs, and effects of glutamate
antagonists were analyzed. It should be kept in mind that this
study used EMG recordings. Thus overall properties are stud-
ied not the details of individual connections.
Reflex network studied

For nociceptive C-fiber-evoked reflexes, which dominate the CO₂ laser responses studied (Bromm et al. 1983; Schouenborg et al. 1992; Weng and Schouenborg 1996a), a network consisting of two interneurons, of which the second-order interneuron, termed reflex-encoder, projects to the motoneurons, is assumed (Schouenborg 2002). The first-order interneurons, thought to be located in substantia gelatinosa, receive a direct input from cutaneous nociceptive C-fibers and often exhibit rather small RFs (Cervero et al. 1979), whereas the reflex-encoders exhibit the same RFs as single muscles in the withdrawal reflex (Schouenborg et al. 1995). Differences in properties for strong and weak connections found in the present study are assumed to reflect properties of connections prior to the reflex encoders because the reflex encoders, but not substantia gelatinosa neurons, exhibit RFs similar to those of the muscles.

Role of inhibitory input in shaping the RFs

In the present study, a detailed analysis of the inhibitory and excitatory RFs showed that, although overlapping to some extent, the organization of the excitatory and inhibitory RFs was clearly different and there was no trace of overlap in the more sensitive parts of the RFs (see also footnote 2). Thus the inhibitory inputs only marginally affect the spatial distribution of reflex gain within the more central parts of the excitatory RF and vice versa. Moreover, the onset latency of the inhibitory input increased toward the excitatory field focus and arrived too late to underlie the differences in onset latency of the excitatory input. Nevertheless, the inhibitory inputs curtail responses from the peripheral part of the excitatory RFs and also limit expansion of the excitatory RFs in high excitability situations (Petersson et al. 2001; Weng and Schouenborg 1996b).

Difference in response properties of excitatory connections with different strengths

Given that inhibitory inputs cannot exclusively explain the gain maps, it follows that differences in the excitatory connections underlie differences in gain. A recent study on the somatotopic organization of the dorsal horn indicates that strong connections simply produce stronger synaptic currents than weak connections (Levinsson et al. 2002). The monosynaptic tactile field potentials in the dorsal horn exhibit a convergence pattern within restricted regions that is similar to that of the withdrawal reflex pathways. For any location within a given region of the dorsal horn, the convergence pattern and weight of input is very similar to a reflex module. Because the spread of synaptic currents is quite limited in the CNS (Mitzdorf 1985; Nowak and Bullier 1996), this would also suggest that the difference between connections of different strength is conserved throughout the region, at least on a macro level. In the present study, the relative reflex gain of different connections was found to be independent of stimulation strength and excitability in each animal tested (Schouenborg et al. 1992). Hence, the order of gain between different skin sites, and thus the spatial distribution of sensitivity, is a very stable feature of this system. The stability of the imprints, per se, may suggest that structural differences underlie relative gain of different connections in this system. For example, the number of primary afferent fibers mediating the input from the skin, the number of substantia gelatinosa neurons contacting reflex-encoders, or the size of synaptic connections, may differ. Structural changes do accompany changes in connection strength in the withdrawal reflex system of Aplysia (Kandel 2001). In that system, the number of synaptic contacts decrease/increase in parallel with behavioral changes in long-term habituation/sensitization protocols.

In the present study, the mean of the maximal differences in onset latency of CO₂ laser-evoked reflex responses differed within the RFs by ∼150 ms. Moreover, the pattern of these onset latencies was very similar to the gain pattern. However, if the stimulation intensity of two different skin sites with different relative gain was matched to produce the same response amplitude, the difference in onset latency was relatively unchanged. Hence, onset latency is not simply a function of response amplitude or stimulation strength. Considering the often low discharge frequency of substantia gelatinosa neurons (Cervero et al. 1979), it is conceivable that the relationship between gain and onset latency reflects the number of substantia gelatinosa neurons contacting a given reflex-encoder neuron as the reflex-encoder may need much longer time for summation of inputs to reach activation threshold. The finding that the relative variance is smaller for sites with stronger gain is also consistent with the possibility that the number of substantia gelatinosa to reflex-encoder connections determines gain and onset latency when the reflex-encoder samples and integrates the activity of many substantia gelatinosa cells representing the
Role of AMPA and NMDA receptors

It has been shown that glutamatergic synapses change their receptor constituents after application of long-term potentiation protocols (Shi et al. 1999). This has spurred great interest in the importance of AMPA receptor cycling. It has been implicated that AMPA receptors are inserted in the action of potentiating a synapse and conversely removed in long-term depression (Galan et al. 2003; Heynen et al. 2000; Ji et al. 2003) and that the ratio of AMPA to NMDA receptors scales with the size of the synapse (Takumi et al. 1999). However, other results indicate that AMPA and NMDA receptors are, in the long term, tightly co-regulated in active synapses, even after synaptic scaling, such that the current ratio is unchanged regardless of synaptic strength (Umemiyä et al. 1999; Watt et al. 2000). Yet it is not known whether these mechanisms are also of importance for the retention of long-term memories (Bailey et al. 2000; Malenka and Nicoll 1999). Moreover, little is known about the role of glutamatergic receptors in shaping the spinal circuits, although NMDA channels seem to have a critical role (Beggs et al. 2002). The present model system is interesting in this context because it offers an opportunity to study the role of glutamatergic receptors in an adult reflex network shaped through learning mechanisms during postnatal development. Indeed, the drug data and simulation of these data are consistent with a co-regulation of AMPA and NMDA receptors in connections of different strength. It should be kept in mind, though, that this conjecture is based on a system level analysis, and information that is more detailed would require experiments at a cellular level.

Importantly, the present results show that the antagonist to NMDA receptors specifically affects the reflex gain whereas the antagonist to AMPA receptors mainly affects the threshold. Hence, it is conceivable that NMDA receptors have a critical role in gain regulation in these pathways. NMDA channels are known to play a role in nociceptive transmission, in particular for central sensitization after injury (Woolf and Salter 2000). Intrathecal applied NMDA antagonists block frequency-dependent potentiation in some of the nociceptive dorsal horn neurons (Dickenson et al. 1997) and in ascending spinal nociceptive pathways to cortex cerebri (Kalliomäki et al. 2003). Moreover, mice with a deficient NMDA receptor complex, due to a deficient L1 adhesion molecule, show a marked hypoalgesia (Thelin et al. 2003). Thus the present results add to the evidence that NMDA receptors have an important role in setting the gain in nociceptive transmission.

Conclusions

The present study shows that connections of different relative gain differ with respect to temporal aspects and response variance. These differences appear to be due primarily to differences in the excitatory connections, although inhibitory connections do contribute to shape the contours of the RFs. It has also demonstrated that NMDA receptors have an important role in setting the gain in the nociceptive system studied. The present study may also suggest that differences in the gain, onset latency, and response variation reflect differences in number of excitatory connections. Further studies at the cellular level are needed to clarify the exact nature of these differences.

APPENDIX: MODELING THE EFFECT OF GLUTAMATE ANTAGONISTS FOR DIFFERENT RECEPTOR POPULATIONS

Model assumptions and parameter values

A simulation of how excitatory pathways with different connection strengths and different AMPA/NMDA receptor populations were affected by NBQX and AP-5 was made to facilitate interpretation of the present pharmacological data. In the simulations, the same basic cellular processes were assumed to be present for all the excitatory

![Figure 7](http://jn.physiology.org/) Effects of glutamate antagonists. A–D: experimental data from 7 animals showing differential effects of glutamate antagonists. A and B: effect of N-methyl-D-aspartate (NMDA) receptor antagonists [or D-2-amino-5-phosphonovalerate (AP-5)] on responses elicited by CO2 laser (n = 5) and electrical stimulation (n = 12), respectively. C and D: effect of AMPA/kainate receptor antagonists [6-nitro-7-sulfamoylbenz(2,3-dione (NBQX)] on responses elicited by CO2 laser (n = 6) and electrical stimulation (n = 13), respectively. Response values under drug treatment were plotted against predrug administration values (···, corresponds to 1:1, corresponding to no effect of the drug).
cells in the pathway. Thus the modulation of the responses in the model can be viewed as taking place at a single cell or being distributed over all the cells in the pathway. It was assumed that the excitatory synaptic transmission was primarily mediated by AMPA and NMDA receptors (You et al. 2003) and that the total charge mediated by the NMDA receptors on opening was three times as large as that mediated by the AMPA receptors (Umemiya et al. 1999). Synapses covering a range of 1–100 receptors of each type were simulated (with no distinction being made between whether they were distributed over a number of synapses or all located in a single synapse).

Two scenarios with different receptor distributions were simulated: 1) A/N (AMPA-to-NMDA ratio) < 1 in weak synapses and > 1 in strong synapses; and 2) A/N = 1 in all synapses. The opening of ≥10 AMPA receptors was assumed to be needed for NMDA receptor activation (this threshold was chosen arbitrarily). The amount of depolarization resulting from activation of the receptors was assumed to directly scale with number of action potentials emitted by the neurons (Reyes 2001) [this assumption was based on the fact that most experiments were done in a highly excitable situation, >3h after spinalization (Schouenborg et al. 1992)]. This can be formally stated as: Response = (N_{AMPA} × 1 + N_{NMDA} × 3) × θ(θ(<0) = 0 and θ(≥0) = 1).

The N_{AMPA} and N_{NMDA} values were chosen to be within a biologically feasible range (Nusser et al. 1998), but altering them did not change the basic outcome of the simulation. Simulations were implemented in MATLAB (Mathwork).

Simulated effect of glutamate antagonists

In the simulations, we assume that the glutamate concentration is similar in different glutamatergic synapses (Auger and Marty 2000). Moreover, we assume that there is no systematic difference in drug concentration, at steady state (Fig. 6), in synapses of different strengths. The latter assumption was based on the findings that AP-5 caused a response decrease almost exactly proportional to the strength of the connections for all sites (Fig. 7, A and B), whereas NBQX application resulted in an absolute decrease of response regardless of site (weaker sites stopped responding) and only a moderate proportional decrease (Fig. 7, C and D).

Under these conditions, the effect of NBQX (competitive AMPA/kainate receptor antagonists) or AP-5 (competitive NMDA receptor antagonists) could be predicted for different hypothetical distributions of AMPA/kainate and NMDA receptors in different reflex connections. In Fig. A1, A and B, it can be seen that the effect of AP-5 is not proportional for connections of different strengths in the first case (Fig. A1A) whereas in the second case it is (Fig. A1B). Furthermore, the effect of NBQX is similar to the experimentally obtained data in the second case. The simulation would thus suggest a co-regulation of AMPA/kainate and NMDA receptors in connections of different strengths.

GRANTS

This project was supported by the Swedish RC Med. Project 01013, the National Network for Neuroscience, Johan and Greta Kochs stiftefel, and the Medical Faculty of Lund.

REFERENCES


Fig. A1. Simulated effects of glutamate antagonists for different proportions of AMPA/kainate (A) and NMDA (N) receptors in strong and weak connections. A: A/N < 1 in weak connections and ≥1 in strong connections. B: A/N = 1 in all connections. —, NBQX 70% block; —, AP-5 70% block; —, all 100 receptors of each type were distributed over a number of synapses or all located in a single synapse.


Nowak LG and Bullier J. Parallel nociceptive re-


