Multiple Spontaneous Rhythmic Activity Patterns Generated by the Embryonic Mouse Spinal Cord Occur Within a Specific Developmental Time Window

Blaise Yvert, Pascal Branchereau, and Pierre Meyrand

Laboratoire de Neurobiologie des Réseaux, Unité Mixte de Recherche 5816, Centre National de la Recherche Scientifique and Université Bordeaux 1, 33405 Talence Cedex, France

Submitted 12 November 2003; accepted in final form 5 January 2004

Yvert, Blaise, Pascal Branchereau, and Pierre Meyrand. Multiple spontaneous rhythmic activity patterns generated by the embryonic mouse spinal cord occur within a specific developmental time window. J Neurophysiol 91: 2101–2109, 2004. First published January 14, 2004; 10.1152/jn.01095.2003. Spontaneous rhythmic activity is a ubiquitous phenomenon in developing neural networks and is assumed to play an important role in the elaboration of mature circuitry. Here we describe the day-by-day evolution of spontaneous activity in the embryonic mouse spinal cord and show that, at a specific developmental stage, 2 distinct rhythms coexist. On embryonic days E12.5 and E13.5, we observed a single type of regularly recurring short spike-episodes synchronized across cervical, thoracic, and lumbar levels. By E14.5, in addition to this motor rhythm, another type of spontaneous synchronous activity appeared, characterized by much longer lasting episodes separated by longer time intervals. On E15.5, these long episodes disappeared. Short episodes were less numerous and more irregular except at the cervical level where a rhythm was occasionally observed. By E16.5, this cervical rhythm became more robust, whereas the lumbar level fell almost silent. Surprisingly, at E17.5, spontaneous activity resumed at caudal levels, now characterized by numerous erratic short episodes. A striking ontogenetic feature of spontaneous activity was the occurrence of long episodes only at E14.5. Although concomitant at all levels of the spinal cord, long episodes displayed different patterns along the spinal cord, with tonic firing at the thoracic level and rhythmic discharge with occasional sequences of left/right alternation at the lumbar level. Thus at E14.5, the originally synchronized network has started to segregate into more specialized subnetworks. In conclusion, this work suggests that ongoing spontaneous rhythms do not follow a smooth evolution during maturation, but rather undergo profound changes at very specific stages.

INTRODUCTION

During ontogeny, neural networks undergo dramatic changes leading to mature functional circuits. Early in development, many neural networks—regardless of their final function—express comparable spontaneous rhythmic episodes involving large populations of simultaneously active neurons. Rhythmic activity is indeed a ubiquitous phenomenon in many developing structures of the CNS (e.g., retina, cochlea, hippocampus, cortex, spinal cord; for review see Ben-Ari 2001; O’Donovan 1999; Pen and Schatz 1999), and has been shown to play an important role in the maturation of neural networks (Shatz 1996). In the rodent spinal cord, numerous studies have shown that such activity occurs during the course of development (Branchereau et al. 2002; Hanson and Landmesser 2003; Nakayama et al. 1999, 2002; Nishimaru et al. 1996; Ren and Greer 2003). Early in development, these activities are usually described as short recurrent bursts of action potentials (about 2–5 s in duration) rapidly spreading over the whole spinal cord. These episodes, involving almost simultaneously the different levels of the spinal cord, can thus be termed “synchronous” by contrast to more spatially segregated activities involving only a part of the whole spinal network. Later in development, embryonic spinal networks tend to lose most of their capability to generate recurrent episodes. Beside these spontaneous rhythms, other rhythmic activities can be induced in the embryonic spinal cord by bath-application of drugs such as 5-HT and/or NMDA. Several studies have indeed described the development of neuronal networks responsible for pharmacologically induced locomotor-like activities characterized by left/right and flexor/extensor alternations (Branchereau et al. 2000; Iizuka et al. 1998; Kudo and Nishimaru 1998; Nakayama et al. 2001; Nishimaru and Kudo 2000). However, the role of spontaneous synchronous rhythmic activity in the emergence of functional locomotor networks remains poorly understood. A necessary first step to address this important issue is to have a precise description of the evolution of spontaneous spinal activities during the whole course of development.

Here, we present the day-by-day evolution of spontaneous embryonic activities generated by the mouse spinal cord between embryonic days 12.5 (E12.5) and E17.5. This ontogenetic time window covers a critical period in the development of the mouse spinal locomotor networks during which side-to-side alternating in activity is established (Branchereau et al. 2000). We show that, during a specific 1-day time window (E14.5), in addition to short spontaneous synchronous bursts, spinal networks exhibit an additional type of episodic synchronous rhythmic activity characterized by long-lasting episodes. Interestingly, although short episodes expressed single synchronous bursts along the cord, the firing pattern within long episodes varied according to the levels of the spinal cord. This suggests that E14.5 may be a key developmental stage in mouse for the segregation of an originally synchronous immature spinal network into multiple functional subnetworks.

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Methods

Experimental paradigm

Experiments were carried out according to protocols approved by the European Community Council and conformed to National Institutes of Health Guidelines for care and use of laboratory animals. Embryos were surgically removed from pregnant OF1 mice (Charles River Laboratories, France) previously anesthetized with ether. Embryonic day 0.5 (E0.5) was defined as the day after the mating night. Spinal cords with the medulla from E12.5 (n = 3), E13.5 (n = 5), E14.5 (n = 11), E15.5 (n = 5), E16.5 (n = 5), and E17.5 (n = 5) embryos were dissected in cooled (10–15°C) Ringer solution (in mM: 113 NaCl, 4.5 KCl, 1 MgCl₂, 17H₂O, 2 CaCl₂, 1 NaH₂PO₄, 25 NaHCO₃, 11 glucose) gassed with a mixture containing 95% O₂–5% CO₂ (pH 7.5) and then placed in a recording chamber continuously perfused (3.5–4 ml/min) with the same Ringer solution and maintained at 31–32°C. All spinal cords considered came from different litters (n = 34 pregnant mice). Spontaneous activity was simultaneously recorded from cervical (C2–C8), thoracic (T2–T11), and lumbar (L1–L6) ventral roots with glass suction electrodes. For E14.5 preparations, both left and right lumbar ventral roots were recorded. Raw signals were band-pass filtered (70 Hz–3 kHz), amplified (≈10,000), and rectified and integrated off-line with a time constant of 0.5 s. All recordings were carried out within 4 h after the end of the dissection.

Spinal cord transection

The spinal cord, lying on sylgard, was prevented from moving by using U-shaped pins. Transections were carefully performed at T4–T8 using microscissors between 2 pins placed at the thoracic level. Recordings were resumed about 5 min after the transection and lasted between 1 and 2 h.

Episode definition

Time intervals during which integrated signals were above baseline level were automatically detected as episodes. When 2 such consecutive episodes were separated by <1 s, they were considered to be the same episode.

Statistical analysis

Unless otherwise stated, statistical analyses were performed using the one-way ANOVA followed by the post hoc Scheffe test on means ± SE.

Index of tonicity

The tonic versus rhythmic pattern of long spontaneous episodes recorded at E14.5 was quantified by computing an index of tonicity for each episode. Integrated signals were low-pass filtered below 0.5 Hz and all local maxima and minima were automatically detected within each long episode. With M and m designating the mean amplitude of local maxima and minima, respectively, the index of tonicity was defined as (M – m)/(M + m). This quantity was close to 1 for highly tonic episodes (M = m) and closer to 0 for rhythmic episodes (M ≫ m).

Phase relationship between left and right bursts

At E14.5, the local phase between left and right lumbar bursts occurring within long episodes was computed as the delay between left and right local maxima divided by the local interburst interval. This quantity was 0 and 0.5 for synchronized and antiphase local maxima, respectively.

Results

Ontogeny of spontaneous lumbar activities

In mice, few studies have described the spontaneous embryonic rhythmic activity generated by the spinal cord. Previous studies (Branchereau et al. 2002; Hanson and Landmesser 2003) have shown that early in development (between E11.5 and E13.5), this activity consists of recurrent short-duration bursts (about 2–5 s), and that later in development (E17.5), it becomes erratic and even disappears in some preparations (Branchereau et al. 2002). These studies did not, however, provide a precise day-by-day evolution of spontaneous activities around the period during which this switch occurs. Hence, in the present study, spontaneous activity was characterized on a day-by-day basis between E12.5 and E17.5 (Fig. 1). At E12.5 and E13.5, we found a single type of motor rhythm characterized by short bursts separated by interepisode intervals of 78.4 ± 15.0 s (mean ± SE) at E12.5 and 125.4 ± 13.5 s at E13.5. By contrast, at E14.5, 2 types of rhythms coexisted. The activity pattern seen at E12.5 and E13.5 became interspersed with another rhythm characterized by a longer episode duration and a slower frequency. The coexistence of these 2 types of episodes can be seen in the bimodal shape of the episode duration histogram at E14.5 (Fig. 1) and also in Fig. 2D. Later in the development, at E15.5 and E16.5, the long rhythmic episodes vanished and only sparse short episodes occasionally occurred. One day later at E17.5, spontaneous activity resumed with numerous erratic short episodes.

The histograms of episode duration provided on the right side of Fig. 1 stress the fact that bimodal distribution exists specifically at stage E14.5, whereas all other stages express unimodal distributions. Based on this bimodal nature of episode duration at E14.5, we further defined 2 categories of episodes: short episodes (<10 s) and long episodes (>10 s). Although short episodes were present at all considered stages, long episodes were detected only at E14.5 (see histograms in Fig. 1 and black bars in Fig. 2, A–C). We found that the duration of short episodes increased from 1.7 ± 0.2 to 4.5 ± 0.6 s between E12.5 and E13.5 (P = 0.009) and was maximal at this latter stage. Short episode duration then decreased at later stages (3.3 ± 0.4 s at E14.5; 1.9 ± 0.2 s at E17.5, P < 0.01). During the course of development, the overall level of lumbar activity increased from stage E12.5 to E14.5, then decreased dramatically at E15.5 and E16.5, and finally resumed at E17.5 (Fig. 2A; see also Fig. 1). To check that the absence of lumbar activity at E16.5 was not attributed to a...
loose suction of the ventral roots, we bath-applied 5-HT (15 μM) at the end of the recording session, which induced strong tonic firing followed by rhythmic locomotor-like activity (data not shown). At stage E14.5, both long- and short-episode rhythms contributed roughly equally to the overall amount of activity. Long episodes (n = 73 over 6 preparations, 18.1 ± 2.2 s duration) occurred with interepisode intervals of 705.7 ± 200.3 s, and short episodes (n = 420 over 6 preparations, 3.3 ± 0.4 s duration) with intervals of 54.2 ± 3.7 s (Fig. 2B). Concerning other stages, only short episodes were recorded. Until stage E14.5, these short episodes occurred regularly every 55–125 s, whereas after stage E14.5, they occurred erratically as indicated by the increase in the coefficient of variation of interepisode intervals (Fig. 2C). Altogether, these data show that at stage E14.5, the mouse spinal cord expresses 2 distinct spontaneous rhythms. Interestingly, these 2 different activity patterns were not totally independent. Indeed, as illustrated in Fig. 2, D and E, the silent interval after a long episode was longer (P < 0.001) than that after a short episode (arrows in Fig. 2D). Thus the occurrence of a long episode delayed the occurrence of the subsequent short episode, indicating an interaction between both types of activity.

Rostrocaudal distribution of spontaneous episodes during the course of development

Early spontaneous activities are generally described as synchronous bursts spreading over large extents of immature structures (e.g., retina, hippocampus, spinal cord). Here, we examined how the spatial extent of spontaneous activities evolved during the course of development. At early stages up to E13.5, short spontaneous episodes appeared synchronously at cervical, thoracic, and lumbar levels of the spinal cord (Fig. 3A). At E14.5 (Fig. 3B), both short and long episodes were recorded at all levels, although a few isolated short bursts occasionally occurred at cervical level (stars in Fig. 3B). However, 1 day later at E15.5, long episodes were absent and spontaneous short episodes were more sparse and no longer expressed systematically at all levels of the spinal cord (compare Fig. 3, A and B with Fig. 3C). Moreover, a rhythmic cervical activity was observed in 2 of 5 preparations. Then at E16.5, lumbar ventral roots became silent (Fig. 3D), whereas the cervical rhythmic activity was more robust and was recorded in all 5 preparations (interepisode interval of 64.8 ± 14.7 s). Thoracic ventral roots were active in 2/5 preparations and silent in 3/5 preparations. This cervical activity was not consistent at stage E17.5 (Fig. 3E). However, at this stage,
numerous short episodes were generated erratically at thoracic and lumbar ventral roots.

We further investigated the spatial origin (cervical vs. lumbar) of short and long episodes at E14.5. As illustrated in Fig. 4A, long episodes were recorded first either at cervical (top left panel and inset on its right) or at lumbar (bottom left panel and inset) levels. Similarly, short episodes could propagate either in the rostrocaudal or in the caudorostral direction (Fig. 4A, right panels). By contrast with previous recordings reporting rostrocaudal delays of several seconds in the embryonic rat spinal cord (Nakayama et al. 1999), we found that these delays were typically <500 ms (Fig. 4A). Our data thus support the idea that both short and long episodes can be triggered at different levels of the spinal cord. To further highlight this point, we recorded at cervical and lumbar levels in 5 E14.5 preparations before and after performing a complete transection at the thoracic level (T4–T8; see METHODS).

Under control conditions (Fig. 4B1), both short and long episodes were synchronous along the spinal cord as described above. At cervical level, short and long episodes were still present after transection (Fig. 4B2, top trace). At this level, the duration and interepisode interval of short and long episodes remained stable after thoracic section, although values became more variable across all 5 experiments (duration: 2.3 ± 0.2 s

**FIG. 3.** Developmental evolution of spontaneous activities across cervical (C), thoracic (T), and lumbar (L) levels of the spinal cord between stages E13.5 and E17.5. Each trace represents 1,000 s of rectified and integrated signals. Synchronous episodes were recorded until stage E14.5. At E14.5 a few isolated short episodes occur at cervical level only (stars). At E15.5, few short synchronous episodes remain, whereas a rhythmic activity started to appear at the cervical level \((n = 2/5 \text{ preparations})\). At E16.5, lumbar (and frequently thoracic) ventral roots were silent, and the cervical rhythm became more robust and could be recorded in all 5 preparations. At E17.5, spontaneous activity resumed at thoracic and lumbar levels. Cervical rhythm was not recorded consistently at this stage.
before section vs. 2.2 ± 0.6 s after section for short episodes and 13.6 ± 0.5 s vs. 14.8 ± 2.4 s for long episodes; interepisode interval: 77.7 ± 15.1 s vs. 107.8 ± 43.1 s for short episodes and 821.2 ± 155.0 vs. 673.1 ± 123.2 for long episodes). The index of tonicity of long cervical episodes also remained stable (0.69 ± 0.05 before vs. 0.67 ± 0.05 after transection). At the lumbar level, short episodes were recorded in all experiments after transection. Regarding these bursts, we did not observe significant differences, either in their duration (2.7 ± 1.1 s before section to 2.0 ± 0.7 s after section) or in the interepisode interval (122.5 ± 33.3 s before section, 105.6 ± 43.3 s after section). Long episodes were observed at lumbar level in 2/5 preparations. In one of these 2 preparations we observed a single long episode 40 min after transection. In the other preparation, long episodes were recorded with an interepisode interval of 777.1 s (see Fig. 4B2, bottom trace), which was in the range of interepisode intervals measured across all 5 preparations before transection (923.2 ± 122.9 s).

For these 2 experiments, the average index of tonicity of long episodes decreased from 0.64 ± 0.06 to 0.46 ± 0.09 at the lumbar level. Finally at this lumbar level, we noticed a nearly statistically significant decrease in the duration of long episodes from 14.2 s before to 11.9 s after transection [n = 2 experiments, t(1) = 6.4, P < 0.1, 2-tailed paired t-test], whereas the duration of short episodes remained unchanged. Altogether, these data confirm the possibility that both long and short episodes may be triggered either at the cervical level or at the lumbar level.

Differences in the temporal pattern of long episodes along the spinal cord

We finally investigated how the temporal structure of long episodes could vary between regions of the spinal cord at the specific stage of development E14.5. We found that long episodes clearly exhibited different patterns of activity between...
the lumbar and thoracic levels, whereas thoracic episodes displayed tonic firing and lumbar episodes (and to a lesser extent cervical episodes) exhibited rhythmic patterned activity (Fig. 5A). This phenomenon was further quantified by computing the index of tonicity (see METHODS) for long episodes. This index was found to be significantly smaller for lumbar than for thoracic levels: 0.62 ± 0.08 for lumbar and 0.88 ± 0.01 for thoracic, P = 0.013 (Fig. 5B), indicating differences in the temporal structure of long episodes along the rostrocaudal axis of the spinal cord. We also determined whether these patterns could also differ between left and right sides of the spinal cord. We investigated the relationship between ipsi- and contralateral rhythmic patterns occurring within long episodes generated at the lumbar level. Within each episode, the phase difference between ipsi- and contralateral bursts was calculated. On average, we found that left and right lumbar ventral roots tended to generate synchronous bursts within episodes (Fig. 5C). However, although less common, episodes displaying a few cycles of clear alternating rhythm could also be seen (Fig. 5A).

**Discussion**

In the present study, we describe the evolution of spontaneous activities during the course of development of the mouse spinal cord. We found that 1) short episodes of spontaneous rhythmic activity occur regularly over the whole spinal cord at early stages (E12.5, E13.5, and E14.5) and become sparser at later stages (E15.5 and E16.5); 2) long episodes occur specifically at E14.5 and involve all recorded levels of the spinal cord; 3) a progressive diminution of activity at the lumbar level and to a lesser extent at the thoracic level occurs from E15.5 to E16.5; and 4) one and a half days before birth at E17.5, spontaneous activity resumes at the thoracic and lumbar levels with the expression of erratic and more spatially segregated short episodes. Altogether, these data indicate that during the course of development, the spinal cord expresses different types of spontaneous activity patterns within specific time windows. Moreover, both activity patterns (short and long episodes) coexist specifically at stage E14.5 but not at the other stages considered in our study.

Although spontaneous synchronous activities have been extensively described in different systems (Ben-Ari 2001; Branchereau et al. 2002; O’Donovan 1999), few reports have suggested that distinct types of such rhythmic activities may coexist at a given stage of development. For example in chick, whereas short episodes have been described early in development (stage E4; Milner and Landmesser 1999), long-duration rhythmic episodes occur later on (stages E7.5–E10; Tabak et al. 2000). In rodents, most studies have reported a progressive increase of the mean duration of spontaneous episodes generated by the spinal cord during maturation (Hanson and Landmesser 2003; Ren and Greer 2003). Our results in mice do indeed show an increase in episode duration between E12.5 and E13.5, but then rather highlight the emergence, at E14.5, of a new rhythm consisting of long-duration episodes that is superimposed on the rhythm consisting of short episodes. In fact, the duration of short episodes was smaller at E14.5 (3.3 ± 0.4 s) than at E13.5 (4.5 ± 0.6 s), and, although it might be misleading to consider that spontaneous short episodes remain of identical nature during the course of development, their duration even continued to decrease progressively at later stages down to 1.9 ± 0.2 s at E17.5 (P < 0.01). Thus the apparent increase in mean episode duration at E14.5 may be attributed to the occurrence of a new type of activity pattern characterized by long episodes at this stage. In rats, previously published data indicate that spontaneous synchronous episodes may also express short and long episodes at E16.5. However, these studies do not emphasize the coexistence of these 2 distinct rhythms and do not describe long episodes in detail. These authors also report that after E16.5, spontaneous activity then becomes erratic and even disappears at E18.5 and E19.5 (Nakayama et al. 1999; Ren and Greer 2003). Given that the gestation period is 3 days longer in rats than in mice, it is likely that stage E14.5 in the mouse corresponds to stage E16.5 in the rat. Here, we underline that this stage is the last one exhibiting rhythmic spontaneous activity simultaneously involving all levels of the spinal cord, and also that it is characterized by 2 spontaneous rhythms.

So far one cannot determine whether these 2 types of activity at E14.5 are generated by common neural networks, or whether they rely on distinct circuits. We addressed this point in 3 ways. First, we found that short and long rhythms interacted with each other, given that after a long episode, the interval to the next short episode was longer than the mean interval between 2 short episodes (see Fig. 2, D and E). This result
suggests that both types of activity may stem from shared or at least interacting networks. In such a case, the silent interval after long episodes may be attributed to a depression of these networks, which is a mechanism that has been proposed to occur in the chick spinal cord (Tabak et al. 2001). Second, we determined whether both types of episodes were systematically initiated at different levels. We found that both short and long episodes could arise first either at the cervical or at the lumbar level (Fig. 4A). Third, we studied the effect of transecting the spinal cord at the thoracic level on the generation of the 2 types of spontaneous activities. We found that transecting the spinal cord at the thoracic level tended to suppress long episodes at lumbar ventral roots in 3/5 preparations, whereas short episodes tended to become more numerous at this level. However, remaining short episodes were still present at the cervical level in all experiments, and long episodes could also be recorded at the lumbar level in 2/5 experiments (Fig. 4B). Thus long episodes were more easily generated at the cervical level. However, by contrast with previous results from rat embryonic spinal cord, suggesting that long episodes can be generated only by the cervical level after thoracic transection (Nakayama et al. 1999), we found that the lumbar spinal cord alone has the ability to generate these activities. We may put forward the following speculative hypothesis to explain why the lumbar region was not always able to generate long episodes. Based on the fact that early descending fibers start to invade lumbar regions around stage E14.5 in the mouse (Ballion et al. 2002) and E17 in the rat (Lakke 1997; Rajaofetra et al. 1992), it is possible that the potentiality of lumbar segments to generate long episodes requires descending innervations. Then, possible age differences between litters (the overnight mating period lasted 16 h) could lead to preparations presenting different maturation of descending tracts, and thus having different abilities to express long episodes at the lumbar level. Whether these 2 spontaneous activities have identical neural origins thus remains an open question, which should be further investigated using detailed mapping of activities along the whole spinal cord using spatiotemporal techniques such as optical imaging (Bonnot et al. 2002a) or multielectrode arrays (MEAs).

One surprising feature of the evolution of spontaneous activities during development is the transient pause in its overall intensity observed at lumbar (and frequently also at thoracic) levels at E15.5 and even more at E16.5 (Fig. 2A). Such a transient silent period in spontaneous activities has been also observed in the mouse retina (Demas et al. 2003), where the ontogeny of spontaneous activity strikingly resembles that reported here in the spinal cord: short recurrent episodes are observed until P13, then long episodes appear at P15, and finally activity weakens around P21 before resuming with a more erratic structure at later stages (compare our Fig. 1 with Fig. 2 of Demas et al. 2003). It is possible that the establishment of synaptic inhibition mediated by chloride ions, which occurs around stage E13.5 in the caudal mouse spinal cord (Branchereau et al. 2002), is responsible for the evolution of the nature of spontaneous activity. The establishment of the inhibitory system could be responsible for less-synchronized spontaneous episodes, and especially the sometimes observed asynchronous bursting within long episodes (see Fig. 5). Then the increase of the overall strength of inhibition could cause the transient pause in spontaneous firing, before other ontogenetic modifications restore activity. Previous studies by Branchereau et al. (2002) have reported that E17.5 spinal cord preparations were not systematically active. Here, all our E17.5 preparations were active. This difference might be attributable to the higher temperature at which recordings were performed in the present study (about 32°C compared to about 26°C in Branchereau et al. 2002).

The present study suggests that stage E14.5 corresponds to an important step in the maturation of the mouse spinal network toward more functional and segregated networks. Indeed, we found that long episodes had different patterns at the thoracic level than at the lumbar level: thoracic episodes exhibited tonic firing, whereas lumbar episodes were rhythmically patterned (see Fig. 5, A and B). The frequency of the intraepisode rhythm was 0.23 ± 0.005 Hz, which was comparable to locomotor-like rhythms pharmacologically evoked by 5-HT at stages E16.5 (0.2–0.25 Hz) or E17.5 (0.3 Hz; Branchereau et al. 2000). Although activity was left/right synchronous on average, spontaneous long episodes at E14.5 occasionally exhibited a few cycles of alternating rhythms between left and right sides of the spinal cord, resembling locomotor-like rhythms pharmacologically evoked using 5-HT at E16.5 and E17.5. These spontaneous embryonic rhythms were about 5 to 10 times slower than spontaneous locomotor-like sequences described in the neonatal mouse (Bonnot et al. 2002b; Whelan et al. 2000). Within-episode alternation, however, could be observed only in a minority of long episodes, and only for a few cycles within the episodes. This is consistent with previous studies on 5-HT–induced rhythms at a similar stage of development around E15 being both synchronous and occasionally alternated (Branchereau et al. 2000). It is possible that an alternating rhythm relies on a precise balance between coexisting excitatory and inhibitory interneurons, and that this balance starts to be established around E14.5 in the mouse. Indeed, given that GABA_A- and glycine-receptor–mediated inhibition starts to develop at E13.5 (Branchereau et al. 2002), it is likely that chloride conductances become less excitatory as the chloride reversal potential begins to move toward a more hyperpolarizing state. One can only tentatively suggest that these spontaneous lumbar rhythms may be the first spontaneous expression of embryonic locomotor-like activities stemming from the future neural circuits responsible for specific motor functions such as hindlimb locomotion [central pattern generators (CPGs); Cazalets et al. 1995; Kjaerulff and Kiehn 1996]. This question remains to be addressed in more detail in a situation where the same spinal networks could be monitored during their maturation from embryonic to adult stages.

In conclusion, the spontaneous activity generated by the embryonic mouse spinal cord undergoes sharp changes during development, with the coexistence, at the specific stage E14.5, of 2 different types of episodic activities. This descriptive work should open on further studies focusing on the respective role of these activities. The long episodes at E14.5 may play a role in the refinement of spinal neural circuitry. Indeed, spontaneous activities are known to be essential for the establishment of neural connectivity (Shatz 1996). Moreover, this type of activity is associated with an increase in intracellular calcium (O’Donovan et al. 1998), which is known to promote axonal growth (Gomez and Spitzer 2000). Thus the long episodes we observed in the mouse at E14.5, which likely induce large calcium influxes, may have critical consequences in the elaboration of neural connectivity. Finally, it would be of interest
to determine whether the temporal structures of the episodes are important for the detailed maturation of specific CPGs or whether they are only the consequences of local differences in cellular connectivity and/or constitution.

ACKNOWLEDGMENTS

We thank Dr. A. Hill for helpful comments on this manuscript, and A.-E. Allain for valuable technical assistance.

GRANTS

This work was supported in part by the Région Aquitaine, the Fondation pour la Recherche Médicale (FRM, Paris), and the Ministère de la Recherche et des Nouvelles Technologies (Paris).

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