Early Social Isolation Provokes Electrophysiological and Structural Changes in Cutaneous Sensory Nerves of Adult Male Rats

Bertha Segura,[1] Angel I. Melo,[2] Alison S. Fleming,[3] Maria Eugenia Mendoza-Garrido,[4] Margarita González del Pliego,[5] Elsa L. Aguirre-Benitez,[5] Jesús Hernández-Falcón,[6] Ismael Jiménez-Estrada[4]

[1] Department of Biology, FES Iztacala, UNAM. Av. de los Barrios 1 Col. Los Reyes Iztacala, Tlanepantla de Baz, Estado de México, CP 54090, México

[2] Centro de Investigación en Reproducción Animal, CINVESTAV Laboratorio Tlaxcala. Universidad Autónoma de Tlaxcala, A.P. 62. C.P. 90000, Tlaxcala, México

[3] Department of Psychology, University of Toronto at Mississauga, Mississauga, Ontario 5L 1C6, Canada

[4] Department of Physiology, Biophysics and Neurosciences, CINVESTAV, IPN Av. Instituto Politécnico Nacional 2508 Col. San Pedro Zacatenco, Del. Gustavo A. Madero, C.P. 07360, México, D.F, México

[5] Departmento de Embriología, Facultad de Medicina, UNAM, Mexico

[6] Laboratorio de Redes Neuronales, Departamento de Fisiología, Facultad de Medicina, UNAM, Mexico

Received 7 February 2014; revised 14 May 2014; accepted 2 June 2014

ABSTRACT: **Sensory and social deprivation from the mother and littermates during early life disturbs the development of the central nervous system, but little is known about its effect on the development of the peripheral nervous system. To assess peripheral effects of early isolation, male rat pups were reared artificially in complete social isolation (AR); reared artificially with two same-age conspecifics (AR-Social); or reared by their mothers and with littermates (MR). As adults, the electrophysiological properties of the sensory sural (SU) nerve were recorded. We found that the amplitude and normalized area (with respect to body weight) of the compound action potential (CAP) response provoked by single electrical pulses of graded intensity in the SU nerves of AR animals were shorter than the CAP recorded in SU nerves from MR and AR-Social animals. The slope of the stimulus-response curve of AR SU nerves was smaller than that of the other nerves. The histological characterization of axons in the SU nerves was made and showed that the myelin thickness of axons in AR SU nerves was significant lower (2–7μm) than that of the axons in the other nerves. Furthermore, the area and axon diameter of SU nerves of both AR and AR-Social animals were significant lower than in MR animals. This is the first report to show that maternal and littermate deprivation by AR disturbs the development of the myelination and electrophysiological properties of axons in the SU nerve; the replacement of social cues prevents most of the effects.** © 2014 Wiley Periodicals, Inc. Develop Neurobiol 74: 1184–1193, 2014

Keywords: **artificial rearing; maternal separation; electrophysiology; sensory sural nerves; myelin**

This article was published online on 17 June 2014. An error was subsequently identified. This notice is included in the online and print versions to indicate that both have been corrected 30 June 2014.

Correspondence to: A.I. Melo (meloangel@gmail.com).

Contract grant sponsor: CONACYT, Grupo de Investigación: G3
Contract grant number: 181334.

Published online 5 June 2014 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/dneu.22197

INTRODUCTION

In mammalian altricial species that show extensive neural development after birth, the social interactions between mother and offspring have a large impact on postnatal development. Mothers' interaction with offspring affects a wide range of physiological, behavioral, cognitive, and sensory processes of pups (Hofer, 1994). In rodents, pup-directed maternal behavior consists of retrieving the pups when they are at a distance from the nest, grouping them, licking them intensely, in both the body and anogenital regions, and nursing (Rosenblatt and Lehrman, 1963; Stern and Lonstein, 2001; González-Mariscal and Poindron, 2002). The natural variation in the frequency and/or intensity of maternal behavior affects the development of individual differences in behavioral and physiological phenotypes of the developing pups. For example, as adults, the biological offspring of mothers that exhibit less licking and grooming and arched-back nursing of pups during the first 10 days of life (in comparison to pups who received high level of maternal stimulation) show lower levels of maternal care towards their own offspring, higher emotionality and activity (Liu et al., 1997; Caldji et al., 1998), deficits in attention (Zhang et al., 2005), and poorer learning (Bredy et al., 2003). Although the relation between maternal phenotype and pup development seems clear, the nature of the effective stimulation provided by the mothers is less so.

In a series of previous studies, we explored the effects on the postnatal development of the offspring of maternal and littermate separation and 'replacement' stimulation, using an artificial rearing system (AR). In comparison to siblings raised by their mothers, pups separated from the maternal nest and artificially reared animals display extensive changes in later maternal, social learning and aggressive behavior (Gonzalez et al., 2001; Melo et al., 2006, 2009). Furthermore, these pups show, as adults, high impulsivity, attention deficits, and disturbed reward processing (Lovic and Fleming, 2004; Burton et al., 2006; Lovic et al., 2011; Lomanowska et al., 2011). These rats also show alterations in a variety of physiological endpoints, including their stress response (Burton et al., 2007; Belay et al., 2011), glucocorticoid receptor density in the hippocampus (Belay et al., 2011), and pattern of dopamine release in the nucleus accumbens (Afonso et al., 2011). Other effects of maternal and littermate separation are observed in the overall structure of neurons, particularly in their dendritic morphology (Shams et al., 2012) and distribution in brain of neural proteins including brain derived nerve factor (BDNF), synaptophysin, and myelin producing protein (S-100) (Chatterjee et al., 2007; Chatterjee-Chakraborty and Chatterjee, 2010). Finally, AR rats also show decreased cell survival in regions of the excitatory limb in the bed nucleus of the stria terminalis and nucleus accumbens (Akbari et al., 2007), and low cFos immunoreactivity in a number of hypothalamic and limbic brain areas, which have relevance for behavior (Gonzalez and Fleming, 2002; Akbari et al., 2008).

However, if pups who are socially isolated (AR pups) are provided with additional licking-like stimulation, of 5–8 bouts a day, or are placed in the environment with other pups, as social stimulation, many of the behavioral and physiological changes brought about by social isolation are partially or completely prevented (Gonzalez et al., 2001; Levy et al., 2003; Lovic and Fleming, 2004; Melo et al., 2006, 2009; Chatterjee et al., 2007; Chatterjee-Chakraborty and Chatterjee, 2010). These AR studies show that many of the behavioral and physiological differences found between low and highly-licked pups can be mimicked in comparisons of pups raised under early social isolation, who receive varying amounts of additional sensory/social stimulation.

The later observations lead to the proposal that sensory and/or other social stimulation from the mother and littermates during early life is of paramount importance for the postnatal development of the individuals and raise the question of how sensory and social stimulation exert their effects and how these stimuli become integrated into the nervous system of the young animal.

One hypothesis that we test in this article is that early social isolation also disturbs the development of peripheral processes involved in the afferent transmission of sensory/social cues from peripheral sensory receptors to the spinal cord and then to the brain. Consistent with this hypothesis, it is possible that the lack of exposure to relevant sensory and social stimuli during AR isolation affects the processes of electrical transmission in the peripheral nervous system (PNS) possibly as a result of deficient myelin formation and/or loss of axons. Hence one route through which social isolation early in life could affect the CNS and its regulation of behavior is by altering the sensory input to the CNS that may be essential for normal development of the CNS.

Myelination by oligodendrocytes in the CNS and Schwann cells in the PNS are essential for providing the membranous insulation that allows for rapid conduction of nerve impulses in large diameter axons. The structure of myelinated axons, including the

architecture of the nodes of Ranvier at which saltatory conduction occurs, is as important as are the ionic mechanisms underlying impulse conduction itself (Dupree et al., 2004). A proper formation of myelin in the axons of peripheral nerves and-or central fiber tracts allows the adequate propagation of impulses from the periphery to the central nervous system and *vice versa*. Inadequate or insufficient myelination results in disrupted impulse propagation, including a decrement in the conduction velocity, intermittent, or unsynchronized transmission or a total blockade in the propagation of action potentials (Waxman et al., 1995; Kimura and Itami, 2009). Such disturbed myelination occurs in axons of the peripheral cutaneous nerves of undernourished animals, which induces an inadequate activation of spinal cord neurons (Segura et al., 2001, 2004; González et al., 2011; Quiróz-González et al., 2012).

It is well known that myelination is a regulated, highly dynamic developmental process that starts during intrauterine life (Tolcos et al., 2011) and continues during the postnatal period (Yakovlev and Lecours, 1967). In addition it has been proposed that the myelination process can also be altered by feedback from impulse activity in axons (Demerens et al., 1996; Stevens et al., 1998) as well as by genetic or external environmental factors such as malnutrition (Segura et al., 2004) or high exposure to toxic substances (García-Chavez et al., 2007). Given that the electrical activity of axons positively promotes the myelination process (Ishibashi et al., 2006) we hypothesized that the deprivation of activation of these axons during early life by social isolation could affect the myelination process, and consequently the electrophysiological functionality of the peripheral nerves.

In the present study, we explored the effect of maternal and littermate deprivation on the electrophysiological properties and myelin thickness of axons in the sensory SU nerves of the AR male adult rats, as compared with their MR siblings.

The SU nerve is mostly comprised of afferent (sensory) fibers that innervates the skin of the feet, sensing diverse sensory modalities such as thermal, touch, light pressure, and noxious stimulus. The SU nerve constitutes an experimental model to study the electrophysiological properties of PNS (Segura et al., 2001, 2004). Since the addition of tactile or social stimulation during AR prevents most of the negative effects on brain and behavior (Gonzalez et al., 2001; Levy et al., 2003; Lovic and Fleming, 2004; Melo et al., 2006, 2009), in the present study we included three groups of animals, an AR minimal stimulation group (AR), their mother-reared siblings (MR), and an additional group of AR animals that were raised with a same-age conspecific (AR-Social).

MATERIAL AND METHODS

Adult female rats (Wistar; $n = 14$) and their male offspring ($n = 42$) from the animal house facility of the "Centro de Investigación en Reproducción Animal" in the Autonomous University of Tlaxcala, México were used. The animals were maintained at a constant temperature ($22 \pm 2°C$) and humidity ($40 \pm 1\%$) under a 14 h/10 h light/dark cycle, with the dark period beginning at 14:00 h, and given Purina rat chow and water *ad libitum*. All procedures were applied in accordance with the guidelines of the Mexican Official Norm (NOM-062-ZOO-1999).

Experimental Procedure

On the day of parturition (day 0), every litter ($n = 14$) was culled to 8 pups: 5 males and 3 females. From the 112 pups obtained 42 males were assigned in this study, with an equal number from each of the 14 litters (3). However, 5 AR-Social males and 4 MR male were not included in the study due to loss of the weight during rearing, or due to a low viability of the nerves during the electrophysiological recording (see below). Hence, the final litter sizes were MR ($n = 10$); AR ($n = 14$). AR-Social ($n = 9$). We used only males to avoid a possible indirect effect of the hormonal status on the electrophysiological properties of nerves that could potentially occur in female rats. On day 3, 3 male pups were taken from each litter and randomly assigned to the following treatment groups: one pup was marked with indelible ink marker and returned immediately to his original litter (mother-reared control group; MR; $n = 10$ male pups). The other 2 pups selected were implanted with a catheter in the stomach (gastrostomy, see below) and reared within an artificial system (AR), in absence of maternal contact, and fed via the catheter with an artificial milk formula diet (Messer et al., 1969; Smart et al., 1983). One of these pups was reared in isolation (artificially reared in complete social isolation; AR; $n = 14$) and the other was reared in the company of 2 pups of the same age (artificially reared, deprived of maternal contact but allowed contact with 2 peers; AR-Social; $n = 9$; See Melo et al., 2006, 2009 for details). The companion pups were obtained from donor litters, randomized with regard to sex, and replaced every 12 h with 2 newly fed pups, in order to allow them to nurse. These pups were not used in the experiment. At postnatal day 22, all pups (AR and MR) were "weaned" and moved to acrylic cages ($34 \times 23 \times 15$ cm^3) and housed with an untreated male of the same age, and continued to be housed in pairs until adulthood. At 5–6 months of age, all males (MR, AR, and AR-Social) were used for the electrophysiological (MR; $n = 6$, AR; $n = 10$, AR-Social; $n = 5$) or

histological (MR; $n = 4$, AR; $n = 4$, AR-Social; $n = 4$) characterization of the SU nerves (see below).

Artificial Rearing Procedure

The artificial rearing protocol was the same as that used in previous studies (Gonzalez et al., 2001; Gonzalez and Fleming, 2002; Lévy et al., 2003; Lovic and Fleming, 2004; Novakov and Fleming, 2005; Melo et al., 2006, 2009). A polyethylene catheter (PE-10; Intramedic Clay Adams) of 10 cm in length was implanted as follows: pups were removed and weighed from the nest at postnatal day 3, and were anesthetized in a bell jar with ~1 to 2 mL of methoxyfluorane (Metofane, CDMV). Inside the catheter was placed a leader wire cable of stainless steel (0.25 mm in diameter) shelter in silastic tubing (Dow Corning, VWR Scientific). The silastic-covered end of the implant, previously coated with vegetable oil, was introduced into the pup's mouth and pushed down the esophagus to the stomach wall, and then pushed through the stomach wall and the ventral surface of the pup's body, to the outside. The rest of the tube was lubricated with oil and pulled gently through the pup until the flanged end contacted the inside wall of the stomach. Neosporin antibacterial cream was applied topically at the site of penetration. The implantation usually took no more than 60 s, and the pups awakened within 3–5 min into an incubator. After the surgical implantation of the gastric catheter, the pups were placed individually into small plastic containers (11 cm diameter, 20 cm tall), whose floor was covered with sterilized wood chips, floated in a temperature controlled water aquarium (water was maintained at 38–40°C). The catheter implanted was connected to another larger catheter (PE-50, Intramedic Clay Adams) and this to a syringe containing milk formula (Messer diet; Messer et al., 1969; Smart et al., 1983). The syringe was attached to a programmable Harvard infusion pump (Harvard Apparatus Syringe Pumps, PHD 2000), which was programmed to infuse milk for 10 min every hour, 24 h daily. In the first day of infusion, the milk formula volume was equal to 33% of pup's body weight, and the amount was increased by 1% each day. Every morning the pups were disconnected from the pump, removed from the cups, weighed and their tubing was flushed with distilled water, and the syringes were replaced with new ones filled with warmed artificial milk. The pump was then reset according to the mean body weight of the pups, and the catheters were reconnected. All pups were stimulated on their ano-genital region 2 times per day for 30–45 s with a warm, wet, fine camel hair paint brush, in order to stimulate urination and defecation. On postnatal day 22 all AR and MR were taken off the pumps, and nest, respectively, weighted and paired up with nonexperimental male rats of the same age, placed in acrylic cages and provided with a mixture of milk formula and chow powder and regular rat chow to allow their transition from liquid to solid food. We assessed that physical growth was not affected by artificial rearing: the weights of the pups did not differ significantly among groups at weaning (data not shown).

Electrophysiological Recording

In adulthood, at 5–6 months of age, mother reared (MR), artificial reared (AR), and AR-Social animals were anesthetized and a segment of the sensory SU nerve of both legs was dissected. At the end of the surgical procedures, the animals were euthanized by an overdose of anaesthetic. The electrophysiological procedures for stimulation and recording of the compound action potential (CAP) in the peripheral SU nerves were performed as described elsewhere (Segura et al., 2001, 2004). In summary, a segment of the SU nerve was placed on a recording chamber filled with saline Krebs solution (NaCl 128 m*M*, KCl 3 m*M*, NaH_2PO_4 0.5 m*M*, $MgSO_4$ 1 m*M*, $NaHCO_3$ 21 m*M*, glucose 30 m*M*) at 37°C. One end of the nerve was drawn into a suction electrode wrapped around an isolated silver wire and connected to a pulse generator. Electrical responses in the nerve were evoked by applying single square current pulses (0.5 ms duration, at different strength intensities) and recorded on the other nerve end with a single suction electrode connected to a low-noise, high-gain amplifier (Grass Model p711), and to an oscilloscope (Tektronix Model D13) and to a personal computer for their analysis. The maximal CAP amplitude was evoked at 3 times the stimulus current strength needed to evoke a barely discernible response in the nerve (threshold, xT). The stimulus current was measured as a voltage drop across a 1000 Ω resistor placed in the return path to ground. The measurement of 2 nerves from AR animals was discarded because it was not steady (by unstable recording conditions) and the maximal amplitude and threshold of the recorded CAP showed a decrement variation of 10% or greater. The CAP parameters determined were the following: Electrical threshold (T), peak-amplitude, area, half-width, initial-latency (Li), peak-latency (Lp), and the length of the nerves was measured (D). Subsequently, the mean conduction velocity of the fastest afferent fibers (maximal velocity: Vm = Li/D), the averaged conduction velocity (Lp = Lp/D) and the normalized CAP area with respect to body weight were calculated. In addition, the fiber recruitment rate of nerves (N) was inferred from the slope determined from an exponential equation derived from the stimulus strength (xT)—normalized CAP area relationship.

Histological Procedures

The histological characterization of axons in the left SU nerve of mother reared (MR), artificial reared (AR), and AR-Social rats was determined. Immediately after dissection nerves were fixed by immersion in Karnovsky solution (glutaraldehyde 1.25%/formaldehyde 1.25%/picric acid 0.03%/cacodilate buffer 0.15 *M*, pH 7.4) for 2 h at 4°C. Tissues were rinsed with the same buffer and post fixed in osmium tetroxide 1%/cacodilate buffer for 1.5 h at 4°C, dehydrated in ethanol 30%, 40%, 50%, 60%, 70%, 80%, 90%, 10 min each, 100% thrice by 10 min each and

Table 1 Electrophysiological Characteristics of Sural Nerves from Control and Experimental Rats

	MR	AR	AR-Social
Rats	6	10	5
Number of nerves	12	18	10
Body weight (g)	489.1 ± 32.4	419.2 ± 10.9*	470.3 ± 15
Electrical threshold (μA)	22.3 ± 7.6	23.2 ± 7.7	26.4 ± 8.2
CAP-Peak amplitude (μV)	2446 ± 445.9	375.6 ± 182.9*	1732.8 ± 652.2^{+}
CAP Area (μV/s)	1350.5 ± 293.5	227.4 ± 121.5*	1252.1 ± 310.3
CAP area/body weight	2.04 ± 0.16	0.58 ± 0.08*	2.48 ± 0.26
Half-width (ms)	0.45 ± 0.1	0.46 ± 0.08	0.54 ± 0.07^{+}
Max. Velocity	57.8 ± 11.4	54.5 ± 8.3	53.3 ± 8.9
Peak velocity	28.4 ± 4.7	27.1 ± 4.3	22.6 ± 3.5^{+}

Mean and standard deviation values of the body weight and electrophysiological characteristics of the CAP evoked in sural nerves of mother reared (MR), artificial reared (AR), and AR-social rats.
*,+ indicates statistical differences between AR and AR-Social with respect to MR groups, respectively (nonpaired Student *t*-test, $p \leq 0.05$).

propylene oxide twice by 10 min each, preincluded in propylene oxide/epoxy resin 1:1 for 24 h, propylene oxide/epoxy resin 1:2 for 24 h and included in the epoxy resin Epon 812 at 60°C. Serial transversal sections from 0.5 μm thick were cut on a microtome (Ultracut Reichert-Jung, Austria Type 65 11 01) and stained with toluidine blue and covered with Entellan resin for light microscopy examination. One of the nerve sections was randomly selected and photographed with a digital camera (AxioCam MRc, Zeiss, Jena, Germany) mounted on an inverted microscope (Axio Observer.D1, Zeiss, Jena, Germany) and the nerve diameter and diameter, area, and myelin sheath thickness of each axon in nerves were measured with the Sigma Scan Pro program (version 4.01, Systat Software, USA) and expressed in μm or μm^2, respectively.

Statistical Analysis

Data are expressed as mean ± SD. Differences in physiological and histological characteristics of SU nerves between MR, AR, and AR-Social groups were established by the Student's *t*-test for nonpaired samples (Welchs correction as needed). $p \leq 0.05$ were considered significant. The statistical analysis was performed by using the GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA).

RESULTS

Body Weight

On the day of the electrophysiological experiment, AR rats had a significantly reduced body weight in comparison to MR rats (−14.3%, Table 1; nonpaired Student *t*-test, $p \leq 0.05$). However, the AR-Social rats did not differ in body weight from either the MR or AR animals (Table 1; Student *t*-test, $p = 0.5$).

Nerve Electrophysiology

Single electrical current pulses of graded strength applied to one end of the SU nerve from MR, AR, and AR-Social rats evoked the excitation of low threshold afferent fibers and its response is recorded at the other nerve end as a CAP (Fig. 1). The electrical threshold of the most excitable fibers in MR-SU nerves was slightly inferior, although not significantly different from that determined for the AR-SU and AR-Social-SU nerves (Table 1; nonpaired Student *t*-test, $p = 0.5$). By increasing the stimulus intensity a proportional increment in the amplitude and area of the CAP until

Table 2 Averaged (±S.D.) Values of the Histological Characteristics of Axons in Sural Nerves from Control and Experimental Rats ($n = 5$ Per Rear Condition)

Rats	*4*	*4*		*4*
Number of nerves	5	5		5
	Number of axons	Axon area	Axon diameter	Myelin thickness
MR	1064 ± 162	97.8 ± 1.1	10.7 ± 0.06	7.2 ± 0.64
AR	999.5 ± 95	57.9 ± 0.9*	8.0 ± 0.07*	5 ± 0.43*
AR-Social	1095 ± 143	70.7 ± 0.8^{+}	8.9 ± 0.05^{+}	6.4 ± 0.62

*,+ indicates statistical differences between AR and AR-Social with respect to MR-SU nerves, respectively (nonpaired Student *t*-test, $p \leq 0.05$).

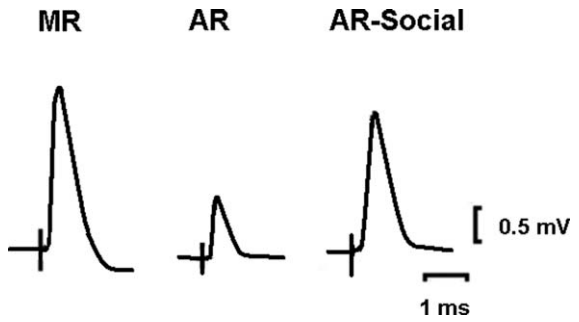

Figure 1 Averaged recordings ($n = 16$) of the CAP evoked stimulus current pulses (3 × T) applied to SU nerves of mother reared (MR), artificial reared (AR) and artificial reared-social (AR-Social) adult rats.

the maximal electrical response in the nerve was evoked, when most of the myelinated fibers were excited (3 × T, Fig. 1). The amplitude and area of the maximal CAP response provoked in MR-SU nerves were not statistically different from those measured in the CAP of AR-Social-SU nerves (Table 1; nonpaired Student *t*-test, $p = 0.5$) and both were significantly larger than the CAP recorded in AR-SU nerves (between 80 and 85%) (Table 1; nonpaired Student *t*-test, $p \leq 0.05$). To exclude the possibility that the CAP area was related to the size or weight of animals, it was determined the normalized CAP area with respect to the animal body weight. Figure 2 illustrates the increments in the averaged normalized area of the CAP provoked in SU nerves of MR and AR and AR-social animals by gradually increasing the stimulus strength (from 1–3 × T). At the different stimulus intensities the CAP evoked in AR-SU nerves was

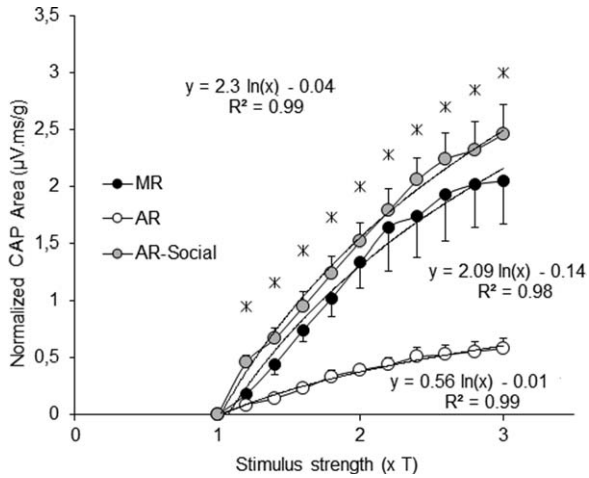

Figure 2 Averaged and standard deviation values of the normalized CAP area of MR (black circles), AR (white circles), and AR-Social (gray circles) SU nerves. Asterisk indicates statistical differences between MR and AR animals (Student *t*-test; $p \leq 0.05$; *n* for each condition was showed in Table 1).

significantly smaller in normalized area than the CAP evoked in MR-SU and AR-Social-SU nerves (nonpaired Student *t*-test, $p \leq 0.05$). In contrast the stimulus intensity-response curve for the CAP evoked in AR-Social-SU nerves was similar to that of the MR-SU curve (Fig. 2) indicating that an equivalent numbers of axons were activated. In concordance, the slope of the stimulus-response curve of MR-SU nerves ($R_{MR} = 2.1$; Fig. 2) was comparable to that of AR-Social-SU nerves ($R_{AR\text{-}S} = 2.3$; Fig. 2) but smaller for AR-SU nerves ($R_{AR} = 0.56$; Fig. 2).

The fastest fibers in the three groups of nerves showed a similar conduction velocity (Table 1) but the fibers responding at the peak of the CAP evoked in AR-Social-SU nerves showed significantly lower conduction velocity than fibers in MR-SU and AR-SU nerves (Table 1; $p \leq 0.05$). In concordance with the latter, the half-width of the CAP recorded in AR-Social-SU nerves was significantly slower than that determined in MR-SU and AR-SU nerves (Table 1; nonpaired Student *t*-test, $p \leq 0.05$).

Nerve Histology

To explore the possibility that the described alterations in generation and propagation of the CAP evoked in SU nerves of AR rats are related to modifications in the number and/or myelin thickness of axons, a histological analysis of MR-SU and AR-SU and AR-Social-SU nerves was made. Figure 3 shows photomicrographs of transversal sections of SU nerves from MR, AR, and AR-Social rats [Fig. 3(A–C), respectively]. As compared to MR-SU and AR-Social-SU nerves [Fig. 3(A,C)], several axons in the AR-SU nerve [Fig. 3(B)] showed degenerative cellular processes, though their number is not significantly different with respect to the other groups of nerves (Table 2; nonpaired Student *t*-test, $p = 0.5$). However, the transversal area (axon area) and myelin thickness of axons in SU nerves from AR rats were significant lower than MR-SU nerves determined (Table 2; nonpaired Student *t*-test, $p \leq 0.05$). Meanwhile the axons in AR-Social-SU nerves showed a less extended reduction in area than MR-SU nerves ($p \leq 0.05$). Figure 4(A) illustrates the frequency distribution of the diameter of axons in the MR-SU, AR-SU, and AR-Social-SU nerves. As compared to MR-SU nerves, AR-SU and AR-Social-SU nerves showed a larger number of small-caliber axons (<5 μm), accompanied with a relative small number of large-caliber axons (>5 μm) (nonpaired Student *t*-test, $p < 0.01$). The frequency distribution of myelin thickness of axons in the SU nerves is showed in Figure 4(B). In this case, axons of SU nerves from AR rats shown a significant reduction

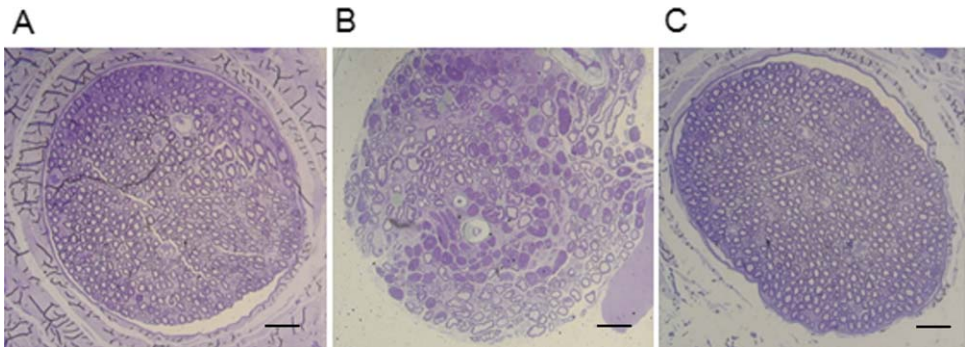

Figure 3 Microphotographs of transversal sections (0.5 μm thick) obtained from sural nerves of (A) MR, (B) AR, and (C) AR-Social rats. Calibration bar: 0.1 mm.

in myelin thickness (2–7 μm) than those contained in the MR-SU and AR-Social-SU nerves (3–9 μm; nonpaired Student *t*-test, $p \leq 0.05$).

DISCUSSION

The experimental evidence derived from the present study shows a considerable depression in the generation and-or propagation of action potentials through axons that comprise the SU nerve of male rats that were deprived postnatally of sensory and social stimulation from their mothers and littermates (AR group), in comparison to their mother-reared siblings. These AR male rats also show a significant reduction in the thickness of the myelin, axon diameter, and axon area of their SU nerve axons. In terms of the effects of additional social stimulation during development, the SU nerve of the artificially reared-socialized animals (AR-Social group) retained most of their electrophysiological capabilities for generation and transmission of action potentials, similar to MR siblings. In concordance with the later, the thickness of the myelin of the SU nerves from the AR-Social animals was similar to the MR animals. These results suggest that the deprivation of sensory and social stimulation during artificial rearing disturbs the development of the sensory SU nerves. Furthermore, the addition of social cues provided from the littermates during isolation can effectively prevent the deficits induced by the maternal deprivation. To our knowledge, this is the first report to show that complete maternal and littermate deprivation early in life disturbs the development of the electrophysiological capabilities of a sensory peripheral nerve and its myelination.

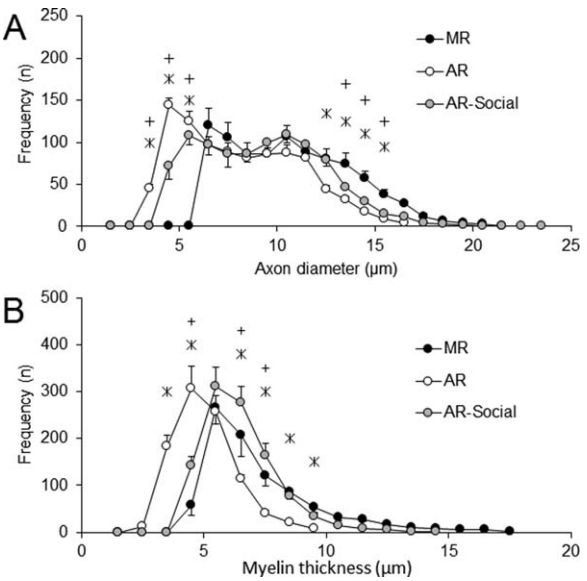

Figure 4 Frequency distribution of the diameter (A) and myelin thickness (B) determined in axons of sural nerves from MR, AR, and AR-Social rats. *,+ indicates statistical differences between MR and AR and MR and AR-Social animals, respectively (nonpaired Student *t*-test; $p \leq 0.05$).

The present results support the proposal that early sensory and/or social stimuli from the mother and/or littermates are involved not only in the development of the CNS, but also in the development of the PNS. These results are consistent with studies showing that mother-infant interactions during postnatal preweaning period participate in the development of myelination (Akbari et al., 2007; Chatterjee et al., 2007; Akbari et al., 2008; Ono et al., 2008), and dendritic morphology in the CNS (Monroy et al., 2010; Shams et al., 2012). It has been reported that early weaning in mice induces a decrease in the diameter of myelinated axons in the anterior part of the basolateral amygdala in young males (Ono et al., 2008), and a deficit in myelin related protein in total brain tissue (Kikusui et al., 2007). Recently, Chatterjee et al. (2007) found that females that were AR show alterations in s-100, a myelin-related protein, which is

"rescued" in AR animals receiving extra tactile stimulation. In addition, other authors have shown that a short period after weaning also is important for the development of the myelination process. For example, social isolation after weaning (8 weeks), reduces the expression of synaptophysin, synapsin I, and myelin basic proteins in the hippocampus and substantia nigra (Lim et al., 2011). Furthermore, partial maternal deprivation (2 h/day between postnatal days 2–14) reduces the total dendritic length and dendritic spine density of neurons in the Prefrontal Cortex, CA1 ventral hippocampus, and nucleus accumbens at a postpubertal age (Monroy et al., 2010).

Here we report the first evidence showing that early sensory/social interaction from the mother/littermates could affect the electrophysiological properties and the thickness of axonal myelin in sensory peripheral nerves. What has been reported thus far is that early maternal deprivation and AR reduced the dendritic length of bulbocavernosus spinal motoneurons (a sexually dimorphic motor nucleus) in correlation with a deficit of penile reflexes (longer latency to erection, few cup erections, and erection clusters) (Lenz et al., 2008). The results obtained in this study suggest an association between inadequate myelination of the fibers in the SU nerve of AR animals and its physiological function, similar to what has been reported for undernourished rats, where the inappropriate myelination of axons in the SU nerve produces a considerable decrease in amplitude and an increased variability in the CAP area (Segura et al., 2001, 2004). In addition, low food consumption between postnatal days 8 and 14 induces a permanent decrease in the expression of myelin proteins, among others the myelin-associated glycoprotein (PLP) and the proteolipid protein MAG (Wiggins and Fuller, 1978; Royland et al., 1993). Although the body weight of AR animals was lower than that of MR animals, in this study we did not find any correlation between the CAP area and the body weight of animals (Fig. 2). These data suggest that the changes observed in the electrophysiological properties, as well as the histological parameters of SU nerves from AR rats is primarily due to the sensory and social deprivation and not to undernourishment.

It is well known that myelination of peripheral axons begins to develop during embryonic life (Gestational days 12–15 in rats), peaks close to birth and slows down by the age of 15–30 days (Mirsky and Jessen, 1996; Jessen and Mirsky, 1998, 1999). Since the postnatal time period when the pups were deprived of the tactile and social stimuli from the mother and littermates coincides with the time period of nerve myelination we assumed that the absence of these stimuli affects the myelination process of peripheral nerves as well as their electrophysiological properties.

Other possible explanations for the nerve alterations observed in this study derive from the idea that sensory stimulation of the skin receptors could trigger the release of several bioactive and/or hormonal components that could be the final mechanisms involved in the development of the peripheral nerves and myelination of central nervous system. For example, it has been reported that giving antisera against nerve growth factor (NGF) to rats during the postnatal days 1–14 causes a complete depletion of high threshold mechanoreceptors conducting in the Aδ range (2–13 m s^{-1}) in the SU nerve, but the same treatment applied from the postnatal day 14 to adulthood had no effect (Lewin et al., 1992). It is possible that this phenomenon occurs in the sensory nerves of AR animals, and that the skin-to-skin contact among the isolated pups and their partners could trigger NGF action in such a way that the negative effects of social isolation could be prevented by social rearing in the cups.

In conclusion, the results presented in this study strongly suggest that the sensory and/or social stimulation that pups receive from their mothers and littermates during early life, promotes the normal development of the structural and functional characteristics of peripheral nerves in the rat. This conclusion is supported by the observed alterations of the thickness of myelin in the axons of sensory nerves and in the CAP evoked in the AR-SU nerves, and the reduction in those nerve changes with replacement of social cues. Such alterations could affect the usual and appropriate propagation of sensory information from the periphery to the CNS, and, as a result, induce changes in the expression of several neural processes and behavior of isolated individuals.

REFERENCES

Afonso VM, King SJ, Novakov M, Burton CL, Fleming AS. 2011. Accumbal dopamine function in postpartum rats that were raised without their mothers. Horm Behav 60:623–643.

Akbari EM, Budin R, Parada M, Fleming AS. 2008. The effects of early isolation on sexual behavior and c-fos expression in naïve male Long-Evans rats. Dev Psychobiol 50:298–306.

Akbari EM, Chatterjee D, Lévy F, Fleming AS. 2007. Experience-dependent cell survival in the maternal rat brain. Behav Neurosci 121:1001–1011.

Belay H, Burton CL, Lovic V, Meaney MJ, Sokolowski M, Fleming AS. 2011. Early adversity and serotonin

transporter genotype interact with hippocampal glucocorticoid receptor mRNA expression, corticosterone, and behavior in adult male rats. Behav Neurosci 125:150–160.

Bredy TW, Humpartzoomian RA, Cain DP, Meaney MJ. 2003. Partial reversal of the effect of maternal care on cognitive function through environmental enrichment. Neuroscience 118:571–576.

Burton C, Lovic V, Fleming AS. 2006. Early adversity alters attention and locomotion in adults Sprague-Dawley rats. Behav Neurosci 120:665–675.

Burton CL, Chatterjee D, Chatterjee-Chakraborty M, Lovic V, Grella SL, Steiner M, Fleming AS. 2007. Prenatal restraint stress and motherless rearing disrupts expression of plasticity markers and stress-induced corticosterone release in adult female Sprague-Dawley rats. Brain Res 1158:28–38.

Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky PM, Meaney MJ. 1998. Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. Proc Natl Acad Sci U S A 95:5335–5340.

Chatterjee D, Chatterjee-Chakraborty M, Rees S, Cauchi J, de Medeiros CB, Fleming AS. 2007. Maternal isolation alters the expression of neural proteins during development: 'Stroking' stimulation reverses these effects. Brain Res 1158:11–27.

Chatterjee-Chakraborty M, Chatterjee D. 2010. Artificial rearing inhibits apoptotic cell death through action on pro-apoptotic signaling molecules during brain development: Replacement licking partially reverses these effects. Brain Res 1348:10–20.

Demerens C, Stankoff B, Logak M, Anglade P, Allinquant B, Couraud F, Zalc B, Lubetzki C. 1996. Induction of myelination in the central nervous system by electrical activity. Proc Natl Acad Sci U S A 93:9887–9892.

Dupree JL, Mason J, Marcus JR, Stull M, Levinson R, Matsushima GK, Popko B. 2004. Oligodendrocytes assist in the maintenance of sodium channel clusters independent of the myelin sheath. Neuron Glia Biol 1: 179–192.

García-Chavez E, Segura B, Merchant H, Jiménez I, Del Razo LM. 2007. Functional and morphological effects produced by repeated sodium arsenite exposure on rat peripheral sensory nerves. J Neurol Sci 258: 104–110.

Gonzalez A, Fleming AS. 2002. Artificial rearing causes changes in maternal behavior and c-fos expression in juvenile female rats. Behav Neurosci 116:999–1013.

Gonzalez A, Lovic V, Ward GR, Wainwright P E, Fleming AS. 2001. Intergenerational effects of complete maternal deprivation and replacement stimulation on maternal behavior and emotionality in female rats. Dev Psychobiol 38:11–32.

González-Mariscal G, Poindron P. 2002. Parental care in mammals: Immediate internal and sensory factors of control. In: Pfaff D, Arnold AP, Etgen AM, Fahrbach SE, Rubin RT, editors. Hormones Brain and Behavior. San Diego: Academic Press, pp 215–298.

González S, Segura B, Guadarrama JJ, Jiménez-Estrada I. 2011. Effect of chronic undernourishment on the cord dorsum potentials and the primary afferent depolarization evoked by cutaneous nerves in the rat spinal cord. Brain Res Bull 85:68–74.

Hofer M.A. 1994. Early relationships as regulators of infant physiology and behavior. Acta Paediatr Suppl 397:9–18.

Ishibashi T, Dakin KA, Stevens B, Lee PR, Kozlov SV, Stewart CL, Fields RD. 2006. Astrocytes promote myelination in response to electrical impulses. Neuron 49: 823–830.

Jessen KR, Mirsky R. 1998. Origin and early development of Schwann cells. Microsc Res Tech 41:393–402.

Jessen KR, Mirsky R. 1999. Schwann cells and their precursors emerge as major regulators of nerve development. Trends Neurosci 22:402–410.

Kikusui T, Kiyokawa Y, Mori Y. 2007. Deprivation of mother-pup interaction by early weaning alters myelin formation in male, but not female, ICR mice. Brain Res 1133:115–122.

Kimura F, Itami C. 2009. Myelination and isochronicity in neural networks. Front Neuroanat 3:1–5.

Lenz KM, Graham MD, Parada M, Fleming AS, Sengelaub DR, Monks DA. 2008. Tactile stimulation during artificial rearing influences adult function and morphology in a sexually dimorphic neuromuscular system. Dev Neurobiol 68:542–557.

Lévy F, Melo AI, Gale Jr BG, Madden M, Fleming AS. 2003. Complete maternal deprivation affects social, but not spatial, learning in adult rats. Dev Psychobiol 43: 177–191.

Lewin GR, Ritter AM, Mendell LM. 1992. On the role of nerve growth factor in the development of myelinated nociceptors. J Neurosci 12:1896–1905.

Lim AL, Taylor DA, Malone DT. 2011. Isolation rearing in rats: Effect on expression of synaptic, myelin and GABA-related immunoreactivity and its utility for drug screening via the subchronic parenteral route. Brain Res 1381:52–65.

Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, et al. 1997. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic–pituitary–adrenal responses to stress. Science 277: 1659–1662.

Lomanowska AM, Lovic V, Rankine MJ, Mooney SJ, Robinson TE, Kraemer GW. 2011. Inadequate early social experience increases the incentive salience of reward-related cues in adulthood. Behav Brain Res 220: 91–99.

Lovic V, Fleming AS. 2004. Artificial-rearing female rats show reduced prepulse inhibition and deficits in the attentional set shifting task-reversal of effects with maternal like licking stimulation. Behav Brain Res 148:209–219.

Lovic V, Keen D, Fletcher PJ, Fleming AS. 2011. Early-life maternal separation and social isolation produce an

increase in impulsive action but not impulsive choice. Behav Neurosci 125:481–491.

Melo AI, Hernández M, Hoffman KL. 2009. Maternal and peer contact during the postnatal period participate in the normal development of maternal aggression, maternal behavior, and the behavioral response to novelty. Behav Brain Res 201:14–21.

Melo AI, Lovic M, Gonzalez A, Madden M, Sinopoli K, Fleming A. 2006. Maternal and littermate deprivation disrupts maternal behavior and social-learning of food preference in adulthood: Tactile stimulation, nest odor and social rearing prevent these effects. Dev Psychobiol 48:209–219.

Messer M, Thomas EB, Galofre A, Dallman T, Dallmen PR. 1969. Artificial feeding of infants rats by continuous infusion. J Nutr 98:404–410.

Mirsky R, Jessen KR. 1996. Schwann cell development, differentiation and myelination. Curr Opin Neurobiol 6: 89–96.

Monroy E, Hernández-Torres E, Flores G. 2010. Maternal separation disrupts dendritic morphology of neurons in prefrontal cortex, hippocampus, and nucleus accumbens in male rat offspring. J Chem Neuroanat 40:93–101.

Novakov M, Fleming AS. 2005. The effects of early rearing environment on the hormonal induction of maternal behavior in virgin rats. Horm Behav 48:528–536.

Ono M, Kikusui T, Sasaki N, Ichikawa M, Mori Y, Murakami-Murofushi K. 2008. Early weaning induces anxiety and precocious myelination in the anterior part of the basolateral amygdala of male Balb/c mice. Neuroscience 156:1103–1110.

Quiróz-González S, Segura-Alegría B, Guadarrama-Olmos JC, Jiménez-Estrada I. 2012. The effect of chronic undernourishment on the synaptic depression of cutaneous pathways in the rat spinal cord. Brain Res Bull 89:97–101.

Rosenblatt JS, Lehrman DS. 1963. Maternal behavior of the laboratory rat. In: Rheingold, HL, editor. Maternal Behavior in Mammals. New York: Wiley, pp 8–57.

Royland JE, Konat G, Wiggins RC. 1993. Abnormal upregulation of myelin genes underlies the critical period of myelination in undernourished developing rat brain. Brain Res 607:113–116.

Segura B, Guadarrama JC, Gutierrez AL, Merchant H, Cintra L, Jiménez I. 2001. Effect of perinatal food deficiencies on the compound action potential evoked in sensory nerves of developing rats. Nutr Neurosci 4:475–488.

Segura B, Guadarrama JC, Pratz G, Mercado V, Merchant L, Cintra L, Jiménez I. 2004. Conduction failure of action potentials in sensory sural nerves of undernourished rats. Neurosci Lett 354:181–184.

Shams S, Pawluski JL, Chatterjee-Chakraborty M, Oatley H, Mastroianni A, Fleming AS. 2012. Dendritic morphology in the striatum and hypothalamus differentially exhibits experience-dependent changes in response to maternal care and early social isolation. Behav Brain Res 233:79–89.

Smart BJL, Stephens DN, Katz HB. 1983. Growth and development of rat artificially reared on a high or a low plane of nutrition. Br J Nutr 49:497–506.

Stern JM, Lonstein JS. 2001. Neural mediation of nursing and related maternal behaviors. Prog Brain Res 133:263–278.

Stevens B, Tanner S, Fields RD. 1998. Control of myelination by specific patterns of neural impulses. J Neurosci 18:9303–9311.

Tolcos M, Bateman E, O'Dowd R, Markwick R, Vrijsen K, Rehn A, Rees S. 2011. Intrauterine growth restriction affects the maturation of myelin. Exp Neurol 232:53–65.

Waxman SG, Kocsis JD, Black JA. 1995. Pathophysiology of demyelinated axons. In: Waxman SG, Kocsis JD, Stys PK, editors. The Axon: Structure, Function and Pathophysiology. New York: Oxford University Press, pp 438–461.

Wiggins RC, Fuller GN. 1978. Early postnatal starvation causes lasting brain hypomyelination. J Neurochem 30: 1231–1237.

Yakovlev PI, Lecours A-R. 1967. The myelogenetic cycles of regional maturation of the brain. In: Minkowski A, editor. Regional Development of the Brain in Early Life. UK: Blackwell Scientific Oxford, pp 3–70.

Zhang TY, Chrétien P, Meaney MJ, Gratton A. 2005. Influence of naturally occurring variations in maternal care on prepulse inhibition of acoustic startle and the medial prefrontal cortical dopamine response to stress in adult rats. J Neurosci 25:1493–1502.