Embryonic amygdalar transplants in adult rats with motor cortex lesions: a molecular and electrophysiological analysis

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INTRODUCTION
It is believed that the function of a neural system results from the high balance and level of specificity of its synaptic connections. The adult brain has a limited capacity for self-repair after neuronal loss caused by trauma or disease. Traumatic alterations of the axonal wiring, such as those happening in cortical lesions, immediately produce a permanent functional impairment which leads to behavioral deficits and have severe anatomical consequences (Sorensen et al., 1989; Plumet et al., 1993; Gaillard et al., 1998; Riolobos et al., 2001; Chen et al., 2002). Embryonic tissue transplanted into the damaged cortex of adult rats has shown successful survival and establishment of reciprocal connections between the host and grafted tissue (Castro et al., 1988; Xu et al., 1991; Plumet et al., 1993; Roger and Ebrahimi-Gaillard, 1994; Frappe et al., 1999; Chen et al., 2002; Gaillard et al., 2007; Gaillard and Domballe, 2008; Santos-Torres et al., 2009) and has lead to behavioral graft-dependent recovery (Labbe et al., 1983; Fernandez-Ruiz et al., 1991; Plumet et al., 1991; Riolobos et al., 2001; Heredia et al., 2004). Therefore, certain level of adequate axonal wiring seems to be necessary for the reconstruction of cortical circuitry and restoration of lost brain function.

Previous studies have demonstrated that homotopic cortical cells transplanted into damaged host adult motor cortex develop into normal cortical neurons and establish functional effective host–graft interconnections (Santos-Torres et al., 2009), suggesting that both events could underlie functional (behavioral) graft-dependent recovery of motor deficits (Plumet et al., 1993; Riolobos et al., 2001). It has also been observed that heterotopic transplants of amygdalar embryonic tissue (a non-cortical and non-motor-related tissue) grafted in adult rats following motor cortex lesion induce the same level of behavioral graft-dependent recovery as homotopic grafts (Heredia et al., 2004), suggesting a similar functional integration of the grafted tissue.

To further the understanding of graft integration and behavioral restoration, numerous immunohistochemical and retrograde axonal tracing studies in the adult brain have been undertaken to...
describe grafted neurons phenotype and determine the nature of graft–host connectivity (Escobar et al., 1989; Castro et al., 1991; Heredia et al., 2004; Thompson et al., 2005; Gaillard et al., 2007; Santos-Torres et al., 2009). However, to our knowledge, molecular characterization of heterotopically transplanted cells with functional restorative capacity has remained virtually unexplored (Su et al., 2009), and electrophysiological studies of how non-cortical grafted neurons are effectively integrated in the adult host brain have also been scarce (Segal and Azmitia, 1986; Castro et al., 1991; Senatorov et al., 1991; Zhou et al., 1998).

The purpose of the present study was to characterize the molecular and functional properties of embryonic grafted amygdaloid cells transplanted into motor cortical locations, by carrying out gene expression studies in parallel with electrophysiological recordings from grafts of amygdalar embryonic tissue implanted into host pre-lesioned motor cortex of adult rats.

Behavioral motor restorative capacity of heterotopic amygdalar transplants into lesioned motor cortex (Heredia et al., 2004) together with the fact that amygdaloid and motor cortical structures share a common ontogenetic origin which might suggest that reestablishment of connections between host cortex and transplanted tissue could be facilitated (Puelles et al., 2000; Medina et al., 2004; Garcia-Lopez et al., 2008) made amygdalar tissue an excellent grafting source candidate.

Besides analyzing astrogliosis induced by graft integration, we provide quantification of the relative mRNA levels of glutamatergic, GABAergic, and muscarinic receptors in the graft and host tissues by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR), and electrophysiological analysis of transplanted neurons and their synaptic afferents coming from host adjacent tissue. Three months after transplantation expression patterns of the receptors were similar in grafted and host control tissues (amygdaloid complex and contralateral motor cortex), and transplanted neurons exhibited normal electrophysiological properties as well as functional synaptic connections coming from the host brain. Our findings show that heterotopic amygdaloid transplanted cells are successfully integrated within the host ontogenetically related cortical tissue and provide a molecular and functional characterization of this integration that could underlie functional recovery induced by this type of transplants.

MATERIALS AND METHODS

All experiments were carried out in accordance with the animal care guidelines of the European Communities Council (86/609/ECC). Twenty three male Wistar rats (Crina Laboratories, France) were used, aged 3 months before surgery. Very briefly, animals were unilaterally lesioned in the motor cortex by aspiration as previously described (Riobolos et al., 2001; Santos-Torres et al., 2009). Seven days after lesion the animals were unilaterally transplanted with donor amygdaloid tissue obtained from 15-day-old rat embryos from pregnant Wistar rats (at 08:00 h on embryonic day 15, E15; sperm-positive vaginal smear DATE = E0). Heterotopic transplantsations of amygdaloid tissue were performed as previously described for homotopic cortical transplants (Riobolos et al., 2001; Santos-Torres et al., 2009). Briefly, donor’s skull was cut at the midline and peeled back, the brain was dissected out and the meninges removed in a dish with sterile glucose–saline. Donor embryonic tissue was set up while the host was being prepared to receive it. Two solid pieces of the embryonic amygdala of approximately 1 mm³ each were taken out from the embryo and located at the bottom of the single motor cortex cavity at the host animal. The cavity containing the transplant was filled up with a piece of gelfoam soaked in glucose–saline and the skin sutured. All experiments were performed 3–4 months after transplantation, all grafts (100%) grew and filled the lesion cavity after this period, and where therefore included in the study.

IMMUNOLABELING

In order to investigate glial scar of the graft, Nissl cresyl fast violet and glial fibrillary acidic protein (GFAP) immunohistological stainings were performed (Figure 1). Deeply anesthetized animals (n = 5) were perfused through the ascending aorta with a wash solution (2% dextan in phosphate buffer, PB 0.1 M, pH 7.4) at room temperature followed by a 4% paraformaldehyde in PB solution. Brains were then removed from the skulls and post-fixed in the same fixative (4°C). Coronal sections were cut at 40 μm, mounted and incubated with the primary antibody solution. A polyclonal rabbit anti-GFAP antibody (Dako; I:1000) was used to visualize astrogliosis. A standard ABC (Vector Laboratories, Burlingame, CA, USA) routine was then followed. The reaction product was visualized with a solution of diaminobenzidine and hydrogen peroxide (Figure 1). Separate sections were stained with cresyl fast violet to determine cortical and transplant cytoarchitecture.

REVERSE TRANSCRIPTASE QUANTITATIVE POLYMERASE CHAIN REACTION

Neurotransmitter receptor gene expression levels were quantified in transplants using RT-qPCR. For tissue collection and RNA preparation, unilateral grafted rats (n = 6) were decapitated and the brains were rapidly removed. Three tissue blocks corresponding to the core of the graft and size-equivalent pieces of both amygdala and contralateral motor cortex were dissected out from each brain under visual control (using a stereomicroscope, Leica, Germany; Figures 1A–C). Graft boundaries were very evident facilitating the dissection procedure. Care was taken not to take any cortical host tissue while the graft-piece was being dissected (briefly, graft was extracted using a micro-spoon, micro-scissors were then used to release meninges, and a sharp blade allowed dissection of the graft core; Figure 1C). Total RNA was extracted from homogenized tissue samples using TRIZOL® (Invitrogen, USA). RNA quality was assessed on a RNA 6000 NanoLabChip (Agilent, USA) and Agilent 2100 Bioanalyzer. Equal amounts (0.4 μg) of total RNA were reversed transcribed using random non-mermers (Sigma, UK), oligo(dT)15 primers (Promega, USA) and SuperScript TM III RT (Invitrogen, USA). Real-time PCR reactions were performed with ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Spain) using Power SYBR Green PCR Master Mix (Applied Biosystems, Spain) with each gene-specific primers (genes coding for GluR1, GluR2, NR1, NR2A, NR2B, GABAaα1 and GABAaα2 subunits, and M1 receptor (M1R) were studied; see below for primer sequences). Control cDNA samples (obtained without transcriptase) were always included, as well as samples without any cDNA template. Efficiencies of PCR
At the end of the qPCR, a melting curve analysis was run to determine the specificity of the products. Sequences and accession numbers are detailed in Table 1.

For electrophysiological characterization of the transplanted neurons in vitro, animals (n = 12) were deeply anesthetized with halothane, decapitated, and standard slicing procedures were performed (see details in Rajeva et al., 1997; Santos-Torres et al., 2009). Sharp electrode recordings were obtained from neurons in coronal slices (n = 3–5 per animal) including the heterotopic graft or the basolateral amygdaloid complex. To morphologically identify the neurons and recording sites in the grafts, some neurons were filled with 2% biocytin (Sigma, UK) in a potassium acetate solution (2 M) at the end of electrophysiological recordings as previously described (Santos-Torres et al., 2009).

All chemicals used in this study were applied by perfusion in artificial cerebrospinal fluid (ACSF). CNQX and APV (Tocris, Spain), AMPA and NMDA receptor antagonists respectively, were added to remove the excitatory glutamatergic responses and atropine sulfate, a muscarinic antagonist (Sigma, UK), was used to study the presence of functional cholinergic receptors. Action potentials and synaptic currents were averaged (n ≥ 5) and expressed here as mean ± SEM.

Statistical analysis of both, molecular and electrophysiological collected data, was performed using two-tailed Student’s t test or ANOVA when appropriate. Statistical significance was determined at a level of p ≤ 0.05.

RESULTS

IMMUNOSTAINING

In order to investigate the cytoarchitecture of the graft and the cellular response of the host to the lesion, Nissl staining and GFAP immunohistological analysis were performed (Figures 1D,E). Amygdalar transplants cytoarchitecture was formed by cellular clusters, as previously described for homotopic cortical grafts (Riolobos et al., 2001), and no cortex stratification was observed (Figure 1D). Transplants included cells with different sizes and number.

Table 1 | Sequences of primers for RT-qPCR.

| Gene | Primer sequence | Gene bank number |
|------|-----------------|-----------------|
| β-Actin | F: 5′-AGCCATGTAGCTAGCCATCC-3′<br>R: 5′-ACCCCATAGTGAGCCACAG-3′ | NM_031144 |
| GluR1 | F: 5′-TCTGGCACCCCTTCTTCCAG-3′<br>R: 5′-CGCATTTCCCTGTTGTTGTT-3′ | NM_031608 |
| GluR2 | F: 5′-CGGCAGCTCAACTAAACT-3′<br>R: 5′-TTGATGCGTGCTGCTTGA-3′ | NM_017261 |
| NR1 | F: 5′-CAGCCGTAAGCTGTGGAAG-3′<br>R: 5′-TGCTCTCACTCCTCTTCTAC-3′ | NM_017010 |
| NR2A | F: 5′-CAGCTGATGTGATATTTTACAGATG-3′<br>R: 5′-ACACTCGTCTATTGCTGACAGA-3′ | NM_02573 |
| NR2B | F: 5′-TCTGGCTTTTCTTATGGATAG-3′<br>R: 5′-CCTCTAGGCGACAGATTAAGG-3′ | NM_02574 |
| GABAa1 | F: 5′-TCTCATGCAGTATGGAACT-3′<br>R: 5′-CTAACGACGGAGAAAC-3′ | NM_183326 |
| GABAa2 | F: 5′-TGGCTGCTTGAACAT-3′<br>R: 5′-GTCCTGGTCTAAGACAGAT-3′ | NM_00135779 |
| M1R | F: 5′-GACCTCATCTTGGGACCTTT-3′<br>R: 5′-GGTCAGGCTGATGACACAGA-3′ | NM_080773 |

F: forward; R, reverse.

ELECTROPHYSIOLOGICAL RECORDINGS IN BRAIN SLICES

For electrophysiological characterization of the transplanted neurons in vitro, animals (n = 12) were deeply anesthetized with halothane, decapitated, and standard slicing procedures were performed (see details in Rajeva et al., 1997; Santos-Torres et al., 2009). Sharp electrode recordings were obtained from neurons in coronal slices (n = 3–5 per animal) including the heterotopic graft or the basolateral amygdaloid complex. To morphologically identify the neurons and recording sites in the grafts, some neurons were filled with 2% biocytin (Sigma, UK) in a potassium acetate solution (2 M) at the end of electrophysiological recordings as previously described (Santos-Torres et al., 2009).

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morphologies. On the other hand, high GFAP expression is one of most distinct characteristics of astrogliosis and therefore, it has been previously reported to be used to study glial scaring (Soares and McIntosh, 1991). As shown in Figure 1E, in heterotopic transplants, graft–host interface was quite notable, showing a very evident glial scar.

GENE EXPRESSION ANALYSIS

To determine whether the expression of neurotransmitter receptors in heterotopic amygdalar transplants was similar to that in cortical and/or amygdaloid host tissues, eight receptor-related transcripts were quantified by RT-qPCR. To monitor glutamatergic, GABAergic, and cholinergic neurotransmission, expression levels of transcripts encoding for (i) the major subunits of ionotropic NMDA (NR1, NR2A, and NR2B) and AMPA (GluR2 and GluR1) glutamatergic receptors, (ii) α1 and α2 subunits of the ionotropic GABAa receptor, and (iii) muscarinic M1 receptor, were investigated in the three brain regions of grafted rats. As shown in Figure 2 the same qualitative mRNA expression pattern was found within the graft and both control regions (contralateral cortex and amygdaloid complex), as the eight receptor subunits and subtypes studied were detected in the three regions analyzed. However, quantification of relative mRNA levels of the eight transcripts showed that all but GluR1 and GABAa2 mRNAs were expressed at significant lower levels in grafted tissue (30–80% less expression) than in contralateral motor cortex (Figure 2A; Glu R1: $t_5 = -1.6, p = 0.19$; GluR2: $t_3 = -3.9, p = 0.01$; NR1: $t_5 = -3.8, p = 0.01$; NR2A: $t_5 = -5.8, p = 0.002$; NR2B: $t_5 = -3.06, p = 0.028$; GABAa1: $t_5 = -3.9, p = 0.026$; GABAa2: $t_5 = -1.9, p = 0.11$; M1: $t_5 = -4.7, p = 0.005$) or the amygdaloid complex (Figure 2B; Glu R1: $t_5 = -1.7, p = 0.14$; GluR2: $t_5 = -2.6, p = 0.04$; NR1: $t_5 = -3.4, p = 0.01$; NR2A: $t_5 = -5.2, p = 0.004$; NR2B: $t_5 = -2.6, p = 0.049$; GABAa1: $t_5 = -2.8, p = 0.038$; GABAa2: $t_5 = -0.24, p = 0.82$; M1: $t_5 = -2.9, p = 0.05$).

INTRINSIC PROPERTIES OF GRAFTED NEURONS

Sharp electrode recordings were obtained from neurons ($n = 20$) in adult grafted rat brain slices. Resting membrane potential (RMP), input resistance (Ri), membrane time constant, and firing threshold were measured. Recorded cells in the transplant were compared with pyramidal basolateral amygdaloid neurons ($n = 8$) of the same preparation. As detailed below, recorded cells in the graft were later on morphologically identified as pyramidal-like neurons using biocytin-labeling techniques.

The direct activation of grafted neurons by supra-threshold depolarizing current injection (0.1–0.5 nA) defined a regular firing pattern of discharge ($n = 10$, Figure 3C), presenting action potentials or a train of spikes with a very slow adaptation (Figure 3C). No differences were found between any of the evaluated parameters in grafted cells (RMP, Ri, mean membrane time constant, and threshold potential) when compared to basolateral amygdaloid neurons [transplant vs. amygdala control: RMP, $-72.1 ± 1.2$ vs. $-71.3 ± 3.1$ mV ($p = 0.81$); Ri, $98.9 ± 19.4$ vs. $102.1 ± 10.4$ MΩ ($p = 0.88$); mean membrane time constant, $18.7 ± 5.9$ vs. $24.3 ± 4.1$ ms ($p = 0.45$); threshold potential, $-52.3 ± 2.6$ vs. $-54.3 ± 2.8$ mV ($p = 0.60$)]. Some of the grafted neurons ($n = 10$) presented spontaneous action potentials at RMP values, as well as basolateral amygdaloid neurons but no significant differences in measured values were found (Figure 3D; transplanted vs. amygdalar cells: spike amplitude, $69.9 ± 1.2$ vs. $73.9 ± 2.1$ mV ($p = 0.12$); rise time, $0.40 ± 0.11$ vs. $0.32 ± 0.02$ ms ($p = 0.56$); decay time, $1.5 ± 0.09$ vs. $1.2 ± 0.2$ ms ($p = 0.15$); half width, $3.5 ± 0.17$ vs. $2.2 ± 1.2$ ms ($p = 0.21$).

EVOKE MED SYNAPTIC RESPONSES IN THE TRANSPLANT

In order to investigate whether connections between adjacent host cortex and neurons in the graft existed, electrical stimuli were applied in the host cortex ($n = 10$), at a distance of 1 mm from the transplant limits (Figure 3A). Excitatory postsynaptic potentials (EPSP) were evoked in all cases indicating successful afferent neurotransmission on transplanted neurons. This synapse presented paired pulse facilitation [$n = 7$; S1 vs. S2 mean values for: amplitude, $6.3 ± 1.2$ vs. $8.6 ± 1.6$ mV ($p = 0.27$); duration, $31.8 ± 10.6$ vs. $42.6 ± 18.4$ ms ($p = 0.62$); rise time, $8.7 ± 1.6$ vs. $10.2 ± 1.3$ ms ($p = 0.48$); decay time, $35.8 ± 7.6$ vs. $38.9 ± 3.1$ ms ($p = 0.71$); and half width, $8.5 ± 2.9$ vs. $10.2 ± 1.2$ ms ($p = 0.59$)] as shown in Figure 3E. The EPSP amplitude showed a graded amplitude.
FIGURE 3 | Electrophysiological properties of amygdalar grafted neurons and characterization of host–graft connections.
(A) Experimental design. Photomicrograph of a coronal section through an amygdaloid transplant into motor cortex stained with cresyl violet. Schematic location of recording and stimulating electrodes is shown. Recordings were made using current clamp technique. Stimuli were applied to the adjacent host cortex. Dashed line indicates transplant edge. T, transplant; Cx: adjacent host cortex. Scale bar 250 μm. M, medial; D, dorsal. (B) A photomicrograph of a 40-μm-thick slice showing a reconstruction of a transplanted neuron labeled with biocytin after intracellular recording. Note the pyramidal morphology of the neuron. Arrow heads indicate neural processes. Scale bar 50 μm. (C) Current clamp responses of transplanted neurons recorded by using sharp electrodes during injection of supra-threshold depolarizing current pulses (0.3 nA, 300 ms) showed a regular spiking firing pattern. (D) Spontaneous action potential recorded from a neuron in the graft and a neuron in the basolateral amygdaloid complex. Note the responses similarity. (E) Graft-evoked synaptic current by adjacent host cortex stimulation. Explored neurons presented a facilitation (136 ± 22%) in EPSP amplitude evoked by the second stimulus (S2) presented to the host cortex in relation to the first (S1), at an interval of 50–100 ms. These evoked responses were blocked by CNQX (10 μM) and APV (50 μM) perfusion. (F) Neuron recorded in the graft showing a sustained response to high frequency stimulation of adjacent cortex. This response was removed by atropine sulfate (1 μM) perfusion.

DISCUSSION

It is well known that astrocytes become activated (astrogliosis) in response to different brain lesions such as trauma, stroke, tumor, etc. Enhancement of GFAP by reactive astrocytes, is the best known hallmark of reactive astrocytes and reactive gliosis (Soares and McIntosh, 1991). The upregulation of GFAP assists in the reconstruction of broken blood–brain barrier (Smith, 2003), and reactive astrocytes produce various neurotrophic factors that assist neuronal survival. On the other hand, astrogliosis causes formation of glial scar which may inhibit axonal regeneration and neuronal connections (Silver and Miller, 2004). Another possible mechanism has been very recently proposed for repairing injured spinal cord (Goritz et al., 2011), which involves unexpected cells, pericytes, that are needed to regain the tissue integrity, and in the absence of this reaction, holes appear in the tissue instead of scarring. In our study, although we found high GFAP expression in transplant–host interface, we could not discard that this new mechanism was involved in transplant integration. Nevertheless, neurotransmission source to grafted cells was enough to maturate functional pyramidal neurons in the transplant indicating that grafting technique proposed here seems to be so effective that the glial scar can not hinder the nerve connections. Hence, our method might be appropriate for graft integration studies.
Our findings clearly show that mRNAs from key receptors are continuously present in the transplant (i.e., qualitative equal expression to controls was found). However, the amount of mRNA detected in the grafts for each subunit/subtype of receptor was lower than in controls, except for GluR1 and GABAα2 subunits. Data presented here are the first to use RT-qPCR to show that the three major excitatory/inhibitory receptor types of the nervous system, glutamatergic, GABAergic, and muscarinic receptors, are present in neural transplants. This confirms previous immunohistochemical and electrophysiological studies in cortical transplants showing glutamatergic (Fricker-Gates et al., 2002; Santos-Torres et al., 2009), GABAergic (Fricker-Gates et al., 2002; Santos-Torres et al., 2009), and cholinergic (Jansen et al., 1997; Santos-Torres et al., 2009) receptors and/or neurons in grafted tissue.

It is well established that neuronal circuits require finely balanced neurotransmission for their definitive formation, as neurons normally die, fail to become fully mature, or show alterations in their physiological properties in the absence of their ante- or retrograde connections. Considering functional integration of the transplant as a consequence of the development of host–graft connections (Castro et al., 1988; Neafsey et al., 1989; Xu et al., 1991; Schulz et al., 1995; Riobolos et al., 2001; Gaillard and Domballe, 2008; Santos-Torres et al., 2009), an adequate synaptic expression of receptors would be needed for restoring connections and lost brain functions following lesions. Therefore, expression of key receptors in the graft, as found here, would be a necessary step to achieve subsequent functional recovery.

As described above embryonic neural grafts placed heterotopically develop a large glial scar 2–3 months after transplantation (Senatorov et al., 1991; Heredia et al., 2004) implying increased number of glial cells and lower neuronal density in the transplant than in the host tissue (see Figure 1A: Petrova and Otellin, 2003). This morphological feature of heterotopic transplants could account for the lower expression levels of most receptor mRNAs found in the graft when compared with both control tissues. In addition, GluR1 and GABAα2 receptor subunits have been reported to be specifically expressed to a significant extent in glial cells (Bureau et al., 1995; Verkhratsky and Steinhauser, 2000; Douyard et al., 2007).

On the other hand, it has been reported that a traumatic brain lesion induces an increase in glutamatergic neurotransmission and then, cellular death mediated by NMDA receptors. Non-NMDA receptors expression also increases so its attenuation might facilitate the functional recovery (Schumann et al., 2008). Hence, a smaller amount of AMPA/NMDA differential expression would reduce cellular death probability induced by excitotoxicity and, at the same time, assures to provide an enough neurotransmission source needed for motor function recovery. In addition, it seems quite obvious that if transplant does not become necrotic and its volume increases, it could mainly be due to an enough source of oxygen and glucose caused by an adequate vascularization (Figure 1E).

In order to confirm if functional integration of the transplant had been reached, we also investigated whether transplanted cells from no cortical origin not only develop similar electrophysiological properties to host pyramidal neurons, but also functional synaptic connectivity from the host cortical tissue. Despite extreme difficulty to obtain recordings from heterotopic amygdalar grafts (mainly due to modified physical consistency of the heterotopic grafted tissue caused by the glial scar), we found that transplanted neurons presented membrane and firing properties similar to those neurons from normal/control amygdaloid complex described in vitro (Chapman et al., 1990; Washburn and Moises, 1992; Yajeya et al., 1997, 1999; Ashenafi et al., 2005). In fact, these cells could not be differentiated by their electrophysiological characteristics from normal pyramidal amygdalar neurons recorded in the same preparation. In addition, biocytin-stained neurons presented a pyramidal-like morphology, as previously reported for cortical grafts (Senatorov et al., 1991). Our results therefore suggest that, at the time of analysis, the transplanted cells had reached a degree of maturation that led them to survive and behave like normal pyramidal cells.

Electrical stimulation of adjacent host cortex elicited glutamatergic and muscarinic synaptic responses in grafted cells, confirming our molecular data and suggesting the presence of functional connections between the host and the transplant. Heterotopic amygdalar transplants in cortical locations connect to adjacent cortex and other structures as thalamus as revealed by neuronal tracer biotin dextran amine (BDA) studies (Gomez-Alvarez et al., unpublished data; Figure A1 in Appendix). This synapse also showed paired pulses facilitation, a synaptic property present in many regions in the brain such as hippocampus (Creager et al., 1980), neocortex (Mercer et al., 2005), or amygdala (Yajeya et al., 2000), that has been related to synaptic plasticity (Nicoll and Malenka, 1999; Madroñal et al., 2009). In addition, the sustained muscarinic response induced in grafted neurons by high frequency stimulation has also been described in amygdala (Washburn and Moises, 1992; Faber and Sah, 2002; Egorov et al., 2006), entorhinal cortex (Egorov et al., 2002), and prefrontal cortex (Mc Cormick et al., 2003), and has been associated with the performance of working memory tasks through recurrent networks. It is well established that persistent activity depends on muscarinic receptors (Major and Tank, 2004; Delgado-García et al., 2006) and it is considered an intrinsic capability for pyramidal neurons in the sensitive and motor cortex (Rahman and Berger, 2011). In addition, cholinergic activity in the motor cortex has been related to synaptic plasticity and motor learning, even showing that a depletion of cholinergic afferents to motor cortex significantly disrupts map plasticity and skilled motor behavior (Commer, 2010). The presence of functional cholinergic afferents on transplanted cells might support the idea that transplant itself would be involved in the recovery of previously learned motor functions. These results suggest that synapses are functionally effective within the host–graft circuitry supporting the hypothesis that amygdaloid cells heterotopically transplanted are successfully integrated within the host cortical tissue, as suggested by its behavioral/functional restoration capacity (Heredia et al., 2004).

Finally, it has previously been reported that the closer the genetic programs of graft and host tissues are, the more adequate the influence of the host is (Senatorov et al., 1991). The genetically determined potentialities remain in the transplanted tissues but will only become manifest if all factors provided by the adequate environment are present (Senatorov et al., 1991). In this regard, a common ontogenetic origin for cortical and amygdalar structures
has been proposed provided by the existence of migrating neurons originating in the ventricular zone of the pallium (cortex) that give rise to the glutamatergic pyramidal neurons within the developing telencephalon (Marin and Rubenstein, 2001). This might explain that grafted amygdalar neural tissue can differentiate into morphologically mature pyramidal neurons assisted by a normal environment supplied by cortical host tissue, receiving functionally effective host projections and exhibiting both normal receptorial expression pattern and electrophysiological properties.

CONCLUSION
In summary this study shows that neural tissue with a non-motor as well as non-cortical origin (amygdala) might be susceptible to be used in attenuating motor deficits and, therefore offers the possibility of using neural tissue from different lines and origins (maintaining the ontogenetic similarity) to recover motor function following irreversible motor cortical lesions in adults. Furthermore, the fact that the present results have been obtained in adult animals (resembling common and difficult clinical scenarios) open new clinical perspectives for brain lesion repair in adulthood, when less synaptic plasticity, more neurodegeneration and delayed functional recovery after ischemic or traumatic surgical lesions is found (Reekmans et al., 2011). Nevertheless, further investigation would be needed to determine the most adequate factors influencing graft integration (Gonzalez-Rey et al., 2010).

ACKNOWLEDGMENTS
This work was supported by the Spanish Ministry of Science and Innovation (grant number BFU2009-07341, SAF2010-14878); MAPFRE Foundation and Spanish Junta de Castilla y León (grant numbers SA092A08 and SA028A09); Lydia Jiménez-Díaz was supported by Juan de la Cierva Programme and Juan D. Navarro-López by Sara Borrell and Ramón y Cajal Programmes. We acknowledge Dr. S. Morcuende and Dr. S. M. Géranton for enlighten comments and the excellent technical assistance of N. González and J. H. Turrión.

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APPENDIX

FIGURE A1 | Heterotopic amygdalar transplants in cortical locations connect to adjacent cortex and other structures as thalamus. Connections between the transplant and the host tissue were revealed by neuronal tracer biotin dextran amine (BDA) injections performed in either the graft (A,a.1,a.2) or ventral and dorsal nuclei of the thalamus (B,b.1,c.1,c.2,D). Eight days after BDA injections, animals were perfused and tissue was cut in 40 μm sections; BDA was visualized with a diaminobenzidine/hydrogen peroxide solution, and sections were then counterstained with cresyl fast violet. (A) Photomicrograph of a coronal section through a heterotopic cortical transplant of embryonic amygdalar tissue. BDA was injected into the transplant. BDA-labeled neurons in the host tissue are shown in (a.1,a.2) at higher magnification [red squares in (A,a.1)]. BDA-labeled fibers within the graft can also be observed in (a.1) (red arrows). (B,C) Photomicrographs of coronal sections through two amygdalar transplants placed heterotopically in motor cortical location. Red squares shows BDA-labeled fibers in the host and the transplant at higher magnification (b.1,c.1). Dashed lines indicate transplant-host interface. Red ovals in (c.1) indicate labeled fibers within the transplant or transplant edge. One of these fibers is illustrated at higher magnification in (c.2). (D) Photomicrograph of a coronal section through the BDA injection site in the thalamus. AVVL, anteroverentral thalamic nucleus; AM, anteromedial thalamic nucleus; Cg1, cingulated cortex, area 1; M1, primary motor cortex; M2, secondary motor cortex; Rt, reticular thalamic nucleus; T, transplant; VA, ventral anterior thalamic nucleus. Scale bars, (A–C): 250 μm; (a.1,b.1,c.1): 100 μm; (c.2): 20 μm.