HIGHLIGHTS FROM THE CENTENARY OF FONDAZIONE MONDINO

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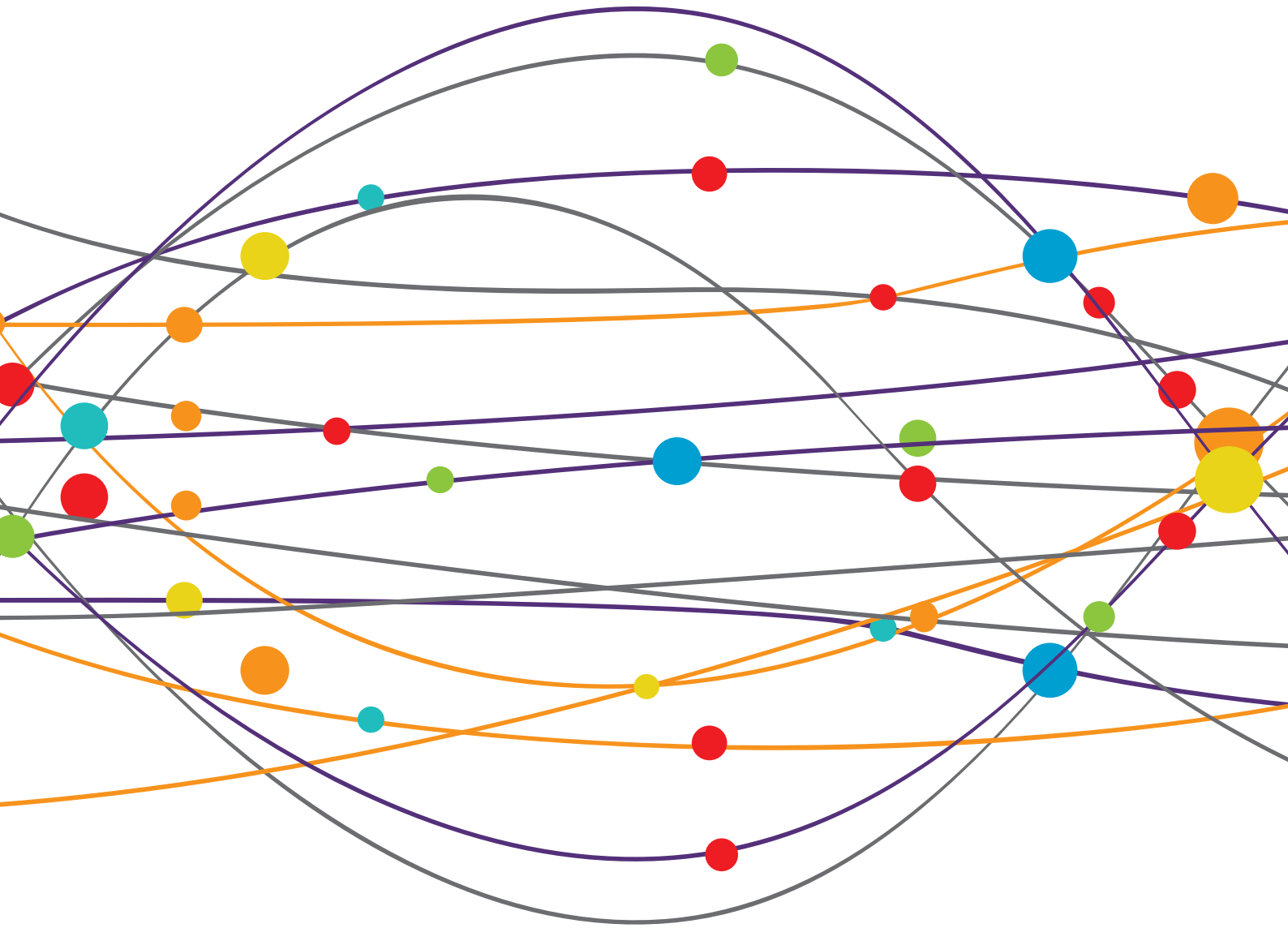

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HIGHLIGHTS FROM THE CENTENARY OF FONDAZIONE MONDINO

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REVIEW
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Is There a Future for Non-invasive Brain Stimulation as a Therapeutic Tool?

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Several techniques and protocols of non-invasive transcranial brain stimulation (NIBS), including transcranial magnetic and electrical stimuli, have been developed in the past decades. These techniques can induce long lasting changes in cortical excitability by promoting synaptic plasticity and thus may represent a therapeutic option in neuropsychiatric disorders. On the other hand, despite these techniques have become popular, the fragility and variability of the after effects are the major challenges that non-invasive transcranial brain stimulation currentlyfaces. Several factors may account for such a variability such as biological variations, measurement reproducibility, and the neuronal state of the stimulated area. One possible strategy, to reduce this variability is to monitor the neuronal state in real time using EEG and trigger TMS pulses only at pre-defined state. In addition, another strategy under study is to use the spaced application of multiple NIBS protocols within a session to improve the reliability and extend the duration of NIBS effects. Further studies, although time consuming, are required for improving the so far limited effect sizes of NIBS protocols for treatment of neurological or psychiatric disorders.

Keywords: neuroplasticity, rTMS, tDCS, NIBS, neuropsychiatric disorders

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INTRODUCTION

Transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) are the most popular techniques of non-invasive transcranial brain stimulation (NIBS).

TMS was introduced in the clinical use in 1985 as a tool to investigate the integrity and the function of human cortico-spinal system (1). Motor evoked potentials can be easily obtained and measured from the contralateral muscles of the stimulated hemisphere; the reproducibility of the responses allowed TMS to become a standard tool in clinical neurophysiology.

The magnetic field produced by TMS easily penetrates the scalp and the skull inducing an electric field in the area just beneath the coil in a painless way (2). The induced electrical field activates the axons of the neurons in the cortex and sub-cortical white better rather than then cell bodies, which have a higher threshold.

TMS produces local effects immediately under the coil and/or remote effects activating axons to or from the site of stimulation. The outcome of such stimulation is quite complex, resulting from a combination of excitatory, and inhibitory effects, that is far away from the organized patterns of activity that occur in natural behaviors.

An alternative way of modulating cortical excitability is to apply a weak current (1–2 mA) to the brain for 5–20 min using a pair of saline-sponged electrodes, one placed over the target cortical area and the other at distance (3). A significant modulation of cortical excitability can be obtained by changing the polarity of the current. Anodal tDCS depolarizes neurons, raising cortical excitability, while cathodal tDCS hyperpolarizes neurons reducing excitability (4).

As we review below, many NIBS protocols can lead to persistent effects on cortical excitability reflecting synaptic mechanisms of long term potentiation (LTP) or depression (LTD). This feature has promoted therapeutic applications in neurological and neuropsychiatric disorders.

In addition, NIBS techniques, have been used as memory and in general as cognitive enhancers.

However, despite the great therapeutic potential of NIBS apart from major depression where TMS has received formal approval from FDA, there is little consensus for a therapeutic use in other neurological and neuropsychiatric conditions (5–7). The reason of this failure is that we need to improve our knowledge on the mechanisms of action of NIBS in order to overcome the variability across individuals (8, 9).

In the present review, will discuss on the mechanisms underlying the neuroplastic effects of NIBS to consider the possible strategies to improve therapeutic effects.

NIBS: AVAILABLE TECHNIQUES

Repetitive Transcranial Magnetic Stimulation

In addition to probing motor cortex excitability with single pulses, TMS can also produce long-term changes in excitability if the TMS pulses are applied repetitively (10).

As a rule, at a regular frequency, low-frequency (1 Hz or less) rTMS reduces cortical excitability (11), whereas high-frequency (5 Hz or greater) rTMS boosts up cortical excitability (11, 12).

This is quite simplistic view, as recent findings have shown that that continuous 5 Hz rTMS decreases instead of increasing corticospinal excitability (13).

rTMS can be delivered in complex burst pattern such as thetaburst stimulation and quadripulse stimulation (QPS) that produce a more reliable effect than conventional rTMS (14–16).

Continuous TBS (cTBS), delivered for 20 or 40 s, decreases cortical excitability, while intermittent TBS (iTBS; applied for 3 min) facilitates cortical excitability. QPS with a short interstimulus interval (e.g., 5 ms) between the 4 pulses facilitates MEPs, while longer interstimulus intervals (e.g., 50 ms) suppress MEPs (16).

Paired associative stimulation (PAS) exploits the principles of Hebbian plasticity imported from animal studies.

PAS was first developed by using low frequency repeated pairing of electrical stimulation of the median nerve combined with TMS over contralateral M1.

Timing is crucial as corticospinal excitability is augmented when the interval between the afferent stimulus and TMS is equal or slightly longer than the individual latency of the cortical N20 component of the median nerve somatosensory-evoked potential. On the contrary, PAS reduces excitability when the interval is shorter than the N20 latency (17, 18).

PAS can also be applied at high frequency (5 Hz), in this case the induction protocol is quite short (2 min) and the intensity of the TMS is under motor threshold. In this way, it is possible to obtain pure cortical effects without effects on spinal excitability (19).

Finally, several variants of PAS protocol have been reported in literature where TMS pulse over the primary motor cortex is paired by another input delivered in remote interconnected cortical areas and even sub-cortical from deep electrodes in patients with deep brain stimulation (20–27).

Transcranial Electrical Stimulation

The techniques widely used in literature are tDCS, transcranial alternating current (tACS), and randon noise stimulation (tRNS) (28).

In tDCS a tiny electrical current (1–2 mA) is delivered over the skull via 2 soaked sponge electrodes. This small amount of current can polarize neurons by changing their firing frequency (28).

Anodal stimulation induces a cortical facilitation whereas cathodal stimulation over the motor area causes suppression (28).

tACS has opened a new window for stimulating the brain at a predetermined frequency with potential therapeutic applications.

It was first developed in animal models applied where tACS can modulate the phase and frequency of discharges in the brain slice models (29). tACS has been used in humans to entrain cortical rhythms via a frequency- specific empowerment as well as to frequency-specific phase realignment of endogenous brain oscillations (30).

Finally, tRNS is another possible way of modulating cortical excitability using a low intensity biphasic alternating current where the frequency varies continuously in a random manner between 0.1 and 640 Hz (full spectrum) or 101–640 (high spectrum) (31).

NIBS AND NEUROPLASTICITY: BASIC MECHANISMS

The after effects induced by NIBS are short lasting (∼30–120 min) in comparison with the long-lasting effects induced in animal model that last for hours to days (32).

Nevertheless, the effect induced with NIBS are more reminiscent of the labile early phase of LTP/LTD (33). In addition, it is likely that other mechanisms are involved such as post-tetanic potentiation (PSP) and short term potentiation (STP) (34).

Transcranial Magnetic Stimulation

Despite TMS-induced plasticity shares certain properties described in animal models, however this assumption must be taken with caution since more direct proofs of physiological mechanisms provided by animal studies are still lacking.

On the other hand, pharmacological manipulation of TMS-induced after effects have shown some features reminiscent of

long term potentiation (LTP) and depression (LTD) described in animal work.

Indeed, it has been reported that dextromethorphan and memantine, glutamatergic antagonist may prevent TMS after effects (35).

Post-synaptic calcium plays a pivotal role in determine whether a glutamatergic synapse is potentiated, depressed, or left unchanged (36).

The role of calcium in TMS induced plasticity has been investigated in different protocols, for instance plasticity induced by PAS and cTBS300 are modulated differently by different drugs acting on voltage-gated Ca2+-channels (37).

As outlined above, although NIBS-induced plasticity shares some properties reminiscent of NMDA glutamatergic plasticity, this assumption should be taken with caution. Nevertheless, *in vitro* studies lead on organotypic preparations have shown that theta rTMS may interact with glutamatergic neurotramsmission even with a structural remodeling of dendritic spines (38, 39).

In addition, rTMS reduces GABAergic strength at dendritic synapses which could represent a permissive factor for inducing subsequent LTP phenomena (38).

Where these synaptic changes do take place at a system level? Epidural recordings do suggest that for instance PAS affects later I-waves, which reflect the activity located not in the cortico-spinal neurons but on the dendritic tree of an excitatory interneuron involved in I3 wave generation (40). For instance, PAS after effects are abolished if later I-waves of the TMS pulse are suppressed by applying a subthreshold conditioning pulse during the protocol (41).

Although the TMS protocols seem to interact with neural plasticity mechanisms we cannot immediately assert that they have a therapeutic role unless we can demonstrate that these artificial paradigms interact with natural behaviors in a useful way. Several studies suggest that this is indeed the case.

TMS-induced changes in motor cortical plasticity interact with learning of simple motor tasks according the rules of metaplasticity.

Metaplasticity is a term used in basic neuroscience describing how synaptic plasticity can be influenced by the previous synaptic history (42). In keeping with the principles of metaplasticity a motor task can change the amount of a subsequent PAS protocol. Indeed, the amount of a facilitatory PAS 25 ms was reduced after a motor task while the after effects of inhibitory PAS 10 ms was increased (18).

Similar effects, reminiscent of metaplasticity, were also described in QPS (16) and TBS (43).

Transcranial Electrical Stimulation

As outlined above tDCS is the most popular technique in clinical practice while tACS and tRNS are more used in a research context (28).

tDCS affects cortical excitability in a polarity-specific manner, anodal stimulation over M1 depolarizes neurons increasing cortical excitability while cathodal hyperpolarizes neurons inducing the opposite effect (3).

However, this vision is too simplistic as duration, strength and direction of the effects also depend on the duration, polarity, and intensity of tDCS. Indeed, a duration of the stimulus above 20 min can reverse the after effect (44, 45).

It is interesting to note that tDCS can modulate the excitability of cortical areas outside M1 such as visual and somatosensory cortices (46, 47).

The mechanisms of action of tACS is still elusive, it has been suggested that it polarizes neurons in a frequency domain through a mechanism named stochastic resonance (48) inducing lasting effects through spike-timing-dependent plasticity (49).

In contrast to transcranial direct current stimulation (tDCS), after effects of tRNS seem to be not NMDA receptor dependent and can be suppressed by benzodiazepines suggesting that tDCS and tRNS depend upon different mechanisms (50).

CURRENT NIBS THERAPEUTIC APPLICATIONS

A group of European experts was commissioned to establish guidelines on the therapeutic use of rTMS from evidence published up until March 2014, regarding pain, movement disorders, stroke, amyotrophic lateral sclerosis, multiple sclerosis, epilepsy, consciousness disorders, tinnitus, depression, anxiety disorders, obsessive-compulsive disorder, schizophrenia, craving/addiction, and conversion (51).

However, there are only 2 conditions where there is a sufficient body of evidence to accept with level A (definite efficacy) the analgesic effect of high-frequency (HF) rTMS of the M1 contralateral to the pain and the antidepressant effect of HF-rTMS of the left dorsolateral pre-frontal cortex (DLPFC) (52).

A Level B recommendation (probable efficacy) is proposed for the antidepressant effect of low-frequency (LF) rTMS of the right DLPFC, HF-rTMS of the left DLPFC for the negative symptoms of schizophrenia, and LF-rTMS of contralesional M1 in chronic motor stroke (53).

Nevertheless, the optimization of stimulation parameters in routine clinical practice in the real world remain to be established.

A level C recommendation (possible efficacy) has been proposed for several conditions:

- LF rTMS of the left TPC on tinnitus and auditory hallucinations;
- HF rTMS (5–25 Hz) of bilateral (multiple) M1 areas on motor symptoms of PD;
- CRPS type I (HF rTMS of M1 contralateral to pain side);
- hemispatial neglect (cTBS of the contralesional left posterior parietal cortex);
- epilepsy (LF rTMS of the epileptic focus), post-traumatic stress disorder (PTSD) (HF rTMS of the right DLPFC);
- cigarette consumption (HF rTMS of the left DLPFC).

In addition, rTMS of DLPFC can be used to empower the effects of antidepressant medication.

Which are the intrinsic mechanisms that permit rTMS and more in general NIBS, to achieve a therapeutic result?

There are two current theories: the "repair model" and the "interactive model."

The first model posits that NIBS may transiently reshape the dysfunction caused by the disease.

Therefore, NIBS, to be effective, should produce persistent changes in brain circuitry. However, at present there is no evidence that this can happen.

The interaction model proposes that rTMS can help the brain restore itself. Within this framework rTMS, or NIBS more in general, may promote or enhance natural adaptations to injury or chronic disease.

Indeed, the plastic effects produced by NIBS, in the offline stimulation mode, may interact with brain network boosting or reducing plasticity phenomena.

Unfortunately, the main limitation is that many of these studies have been conducted on small scale and have only been performed at a single center, being difficult to evaluate.

The only clinical entity where NIBS is widely recognized as treatment is depression, where large clinical trials have been conducted.

The initial clinical trials of rTMS began more than 20 years ago with investigations in patients with drug-resistant depression (54, 55).

The idea of using rTMS was driven by functional imaging evidence showing that patients with depression have reduced activity in the left pre-frontal cortex (56, 57).

Therefore, the strategy was to enhance the activity of pre-frontal cortex with high-frequency stimulation and to prolong the after effects by applying rTMS during several daily sessions.

Regarding pain, several review and meta-analyses (58–63) suggest that high frequency stimulation of M1 contralateral to the pain side can reduce pain (pain relief >30% in 46–62% of patients and >50% pain relief in 29%).

The efficacy of a single HF rTMS session tends to persist for a few days and may be enhanced and prolonged with session repetition, while optimal stimulation parameters need to be better defined. In addition, the role of rTMS in the therapeutic armamentarium against neuropathic pain remains to be established. On the other hand, it has been shown that HF rTMS of M1could predict the outcome of epidural motor cortex stimulation (EMCS) (64–68). However, rTMS tests can be used only to confirm the indication of EMCS therapy but not to exclude patients from implantation (56, 58).

In keeping with the interaction model (see above), rTMS acts as a relatively non-specific input promoting synaptic plasticity during physical therapy sessions.

There are 3 post-stroke disorders which may benefit cortical stimulation techniques: motor deficit, aphasia and hemineglect.

The strategy is to increase the excitability of the ipsilesional hemisphere or to decrease the excitability of the contralesional hemisphere, which results in a reduction of its inhibitory influence onto the lesioned hemisphere that can promote recovery.

However, it is important to note that this might be a simplistic interpretation of the effects of these protocols since the contralesional hemisphere may play in some patients an adaptive role promoting recovery (see below).

A meta-analysis of the literature shows that an increase in excitability produced by HF rTMS of ipsilesional M1 or a decrease in excitability induced by LF rTMS of contralesional M1 tends to improve motor abilities in stroke patients (Levels B or C recommendations).

On the other hand, there are several unsolved clinical issues. First the therapeutic value of either modality of stimulation remains to be determined with respect to the phase of stroke recovery (acute or sub-acute vs. chronic). Second, the real impact of rTMS in daily practice is still unknown. In addition, there are safety concerns regarding the possible risk of seizure increasing cortical excitability at the site of injury.

On the other hand, the systematic use of LF rTMS to reduce the hyperactivity of the contralesional hemisphere must be considered with caution, because the hyperactivity of healthy hemisphere may be sometimes adaptive and this may promote stroke recovery (69–71).

Therefore, the feasibility of rTMS in long term stroke rehabilitation remains to be determined and we are still far from a daily practice use of rTMS.

A key limitation is perhaps the use of generic, unvarying methodology given the heterogeneity that is characteristic of stroke (72). Therefore, the future in the use of NIBS in stroke would be to better understand the pathophysiological mechanisms and stratify patients for tailored or personalized cortical stimulation therapies (73).

Another application of TMS as therapeutic tool in neurology is on migraine. It has been reported that early treatment of migraine with aura by single pulse TMS resulted in increased freedom from pain at 2 h compared with sham stimulation, and absence of pain was sustained 24 h and 48 h after treatment (74). These results have been confirmed by another subsequent study (75). Based on these findings the US Food and Drug Administration (FDA) in 2017, approved a device capable of delivering a single pulse TMS to relieve pain caused by migraine headaches that are preceded by an aura—a visual, sensory or motor disturbance immediately preceding the onset of a migraine attack.

Finally, rTMS can be used also in pediatric neurology with promising results in different clinical situations such as autism spectrum disorders, attention-deficit/hyperactivity disorder, epilepsy, and cerebral palsy (76).

Yet, most clinical TMS and tDCS studies have been published on adult populations, and extensive research into the clinical utility of TMS and tDCS in pediatrics remains an unmet needed.

Indeed, further research is required to investigate the effects of age-related differences in basic neurologic mechanisms on the safety and efficacy of brain stimulation in the pediatric brain.

In the next session we will discuss the limitations of currently available NIBS techniques (69, 77).

VARIABILITY OF NIBS-INDUCED EFFECTS

NIBS after-effects are quite fragile and variable both within and between subjects and this can potentially flaw therapeutic applications. This variability is probably the result of several factors.

Such inter- and intra-individual variability will severely hamper the clinical use of NIBS as a potential treatment of

neurological or psychiatric disorders, therefore the underlying reasons for these variabilities need urgently to be explored (70).

Effect of Voluntary Contraction

TBS after effects are abolished by the contraction of the target muscle, interestingly muscle contraction after cTBS shifts the depression into facilitation while the facilitatory effects of iTBS are enhanced (71).

The nature of the contraction (i.e., tonic vs. phasic) can also influence the after-effects of TBS (76).

Tonic contraction can also influence tDCS reversing the effects of anodal and cathodal stimulation (78) while tonic activation immediately after tDCS abolishes all after effects (79).

Similar effects of voluntary contractions where observed in QPS, where rhythmic hand opening-closing at 1 Hz for 1 min abolishes any effect on corticospinal excitability (80).

The vulnerability of NIBS effects by muscular pre-contraction may be explained by metaplasticity (see above) (81).

Inter and Intra-Subject Variability

Several studies lead in large cohort of healthy subjects have shown a considerable inter- and intra-individual variability in response to all NIBS protocols.

It has been reported that only half of the subjects tested can be considered as responder to TBS protocols (82). Similarly, Wiethoff and associates reported that only ∼50% of subjects could be considered as responders (83).

The response rate of PAS in a large cohort multicentric study lead in Germany was 53% (84).

All together these data suggest that the probability of producing the "expected" response may be lower than 50%, in most NIBS plasticity-inducing protocols.

QPS, a newer form of rTMS, may provide a reduction of variability, with a responder rate ranging from 60 to 80% (85, 86).

In general, the session-to-session, intra-individual variability, is lower than inter-individual variability.

Lopes Alonso and associated reported that about 70% of subjects maintained reproducible responses to anodal tDCS in separate sessions; a similar percentage was reported in another study (9, 87).

Such inter- and intra-individual variability has severely compromised attempts to use NIBS for treatment of neurological or psychiatric disorders. Therefore, future studies are needed to address the reason of such variability to find new strategies to improve NIBS after effects (70).

FACTORS INDUCING NIBS VARIABILITY

Several factors may underlie such a variability and many of them cannot be changed such as age, gender and genetic polymorphisms. Therefore, it is important to control them through the experimental design (88).

Individual brain anatomy can be a potential source of variability especially if we refer to scalp measurements. This can now be corrected by using TMS neuronavigator systems which use individual anatomical brain images to guide the placement of the coil over the region of interest (89).

Another important factor, which is difficult to control, is that the level of ongoing cortical activity interacts with NIBS after effects.

A possible strategy to control neural activity would be to monitor physical activity since it is well-known that it influences NIBS after effects by acting on ion channels. Thus, keeping the subject relaxed could be a way to reduce NIBS variability (90).

Induced cortical activity on purpose may be used to modulate NIBS after effects.

For example, prior muscle pre-contraction may enhance the inhibitory effects of cTBS (91).

This metaplastic effect can be replicated also outside the primary motor cortex; for instance a cognitive task modulating frontal theta wave activity enhanced the antidepressant effect of rTMS (92).

The level of ongoing cortical activity and even its prior history interacts with the effects of NIBS.

The subject attentional focus may profoundly influence NIBS after effects. For instance, PAS after effects are maximized if subject focused on the stimulated hand while the effects are decreased if the subject directed attention on the non-stimulated hand (93).

Menstrual cycle can affect cortical excitability and plasticity; for example rTMS after effects are maximal on day 14 since estradiol reinforces synaptic potentiation by acting on voltage-gated sodium channels (94).

It is also well-known that PAS after effects are lower in the morning in relationship to the circadian rhythms of cortisol and melatonine (95).

Another potential source of variability is represented by genetic factors. It is well-known that subjects carrying the a Val66Met polymorphism in the gene encoding brain-derived neurotrophic factor (BDNF) have a reduced responsivity to NIBS protocols and an altered use dependent plasticity (96). All these factors need to be considered when NIBS is used for therapeutic purposes.

IS THERE A FUTURE FOR THERAPEUTIC NIBS?

The past 20 years have seen the publication of a remarkable number of papers about the potential therapeutic effects of NIBS in conditions ranging from cocaine addiction to stroke and depression.

On the other hand, despite this has stimulated a tremendous amount of research the overall clinical effects are limited except perhaps for depression (5, 51). Therefore, it is necessary to find new strategies to empower the NIBS therapeutic after effects.

Improving NIBS Variability

In the previous section, we analyzed the possible sources of variability of the NIBS after effects that can be controlled to optimize NIBS protocols.

An important variable that need to controlled is the adaptation of NIBS based on the individual brain anatomy. Advances in physics and computational science will allow to design brain

modeling considering the NIBS-induced electrical field, based on bone thickness with local thinning, CSF volume, and gyral folding of the individual brain (97, 98).

For the last 30 years, NIBS techniques, have approached the brain as a black box, ignoring its endogenous excitability at the time of stimulation.

Indeed, there are several evidences pointing out that NIBS effects are state-dependent on a time scale of minutes to hours, depending on the immediate history of neural activity (99) and synaptic plasticity (100). Brain activity changes, on the time scale of seconds to milliseconds, are governed by rhythmic fluctuations in neural excitability within the ascending thalamo-cortical systems and cortico-cortical projections (101, 102). Therefore, NIBS protocols should be optimized not only on neuroimaging data to account for individual differences in functional neuroanatomy but also taking into account the current oscillatory brain state (100).

Perhaps the most promising strategy to enhance NIBS after effects is to monitor neural activity in real time using EEG an then triggering TMS pulses only at pre-defined states (103).

This approach is called state-dependent brain stimulation (BSDBS).

Indeed, the recent advances in combining TMS with EEG have made possible designing stimulation protocols that are controlled by the EEG signal adjusting stimulation in a very direct way, short-circuiting the motor-sensory loop (51).

Although this state dependent approach has a strong theoretical background coming from animal studies, there are only limited evidences that the same synaptic rules can be successfully applied on the human side (104). Nevertheless, EEG brain-state triggered NIBS-in-the-loop set-ups will enable physicians, in the near future, to interfere with their patients' ongoing brain activity with high temporal, spatial and spectral precision.

This approach has several important advantages. Firstly, neuromodulation can be tailored to each patient thus reducing the inter-individual differences in the excitability and connectivity of brain networks (105).

Secondly, monitoring EEG it is possible to detect the time-course of dynamic changes during network reorganization such as during stroke rehabilitation (106).

Thirdly, EEG brain-state triggered NIBS should be recommended since the modifiability of neurons and networks is a function of their recent activity (metaplasticity) and hence this can determine the direction, extent and duration of after effects in neural networks (107).

Finally, another strategy under study is to use the spaced application of multiple NIBS protocols within a session to improve the reliability and extend the duration of NIBS effects.

Traditionally NIBS protocols are delivered or in a single session (once a day) or in multiple sessions (once a day for consecutive days). Hence spaces application could open a new therapeutic window improve the reproducibility of NIBS effects.

There are new evidence suggesting that this spaced approach may be successful.

For instance, the application of two spaced sessions of cTBS over the cortical frontal eye field region increased saccadic eye movement latency for a significantly longer period than a single cTBS protocol (108).

Similarly, spaced stimulation of parietal cortex contralateral to the stroke improves significantly symptoms of visual neglect (109).

Same effects have been reported after the spaced application of tDCS with a significant enhancement of the after effects when the second tDCS application is delivered while the effect of the first tDCS application is still ongoing (110, 111).

Despite these encouraging results of spaced stimulation, the rules governing this new type of stimulation need to be further investigated in future studies.

Indeed, the mere increase in the train duration with several forms of NIBS including TBS (112) and tDCS (45) can reverse the direction of the induced plasticity or abolish the effects (86). At the same time, intensity increase does not necessarily implies an increase in amplitude but may even reverse inhibition into facilitation (44).

Finally, another possible approach is to use pharmacological neuromodulation by varying dopamine, noradrenaline, acetylcholine or serotonin neurotransmitters to empower NIBS induced plasticity.

This is a stimulating new perspective to empower clinical NIBS effects that however have not yet been investigated systematically.

Among the strategies discussed above, spaced application of multiple NIBS protocols is perhaps the most viable tool to empower clinical efficacy of NIBS effects.

However, it is mandatory in future studies, to identify the optimal spacing between stimulation (in the single session) and to run separate studies to improve the after effects using a multisession approach.

Such studies, although time consuming, will be particularly important for improving the so far limited effect sizes of NIBS protocols for treatment of neurological or psychiatric disorders.

Finally, a professionally-supervised protocol for home-based, remotely-supervised tDCS, supported by specially designed equipment and a telemedicine platform, has shown feasibility in research settings. This approach shows promise for reducing patient burden and enabling longer duration of treatment in addition with home telerehabilitation. Indeed, if patients can safely apply tDCS to themselves at home, combining telerehabilitation with tDCS, this approach would be a good opportunity to empower therapy without costly therapeutic face-to-face supervision. For instance, tDCS combined with cognitive training delivered at home induced a better cognitive outcome in comparison with patients who received just the cognitive training alone (113).

This study showed the feasibility of remotely supervised, at-home tDCS and set up a protocol for safe and reliable delivery of tDCS for clinical studies (114).

Recommendations for Future Clinical Trials

In future studies, special emphasis should be given to improve the quality of clinical trials testing the therapeutic efficacy of NIBS;

Several recommendations should be considered in future studies:

(i) increase the sample size and use of realistic placebo control and double blinding in keeping with the rules of drug clinical trials;
(ii) use of randomized cross over study designs and advanced statistical methodologies such as cluster analysis.
(iii) improve anatomical targeting by using neuronavigated TMS;
(iv) encourage new research to discover new feasible targets of stimulation;
(v) increase the dosage of stimulation which is rather low across studies;
(vi) systematic use of priming strategies to empower NIBS after effects;
(vii) defining and improving clinically valid endpoint measures.

Nevertheless, it should be reminded that rTMS could be used as preoperative predictive factor for selecting candidates for surgery and to validate the cortical target of where to implant electrodes for epidural invasive stimulation.

Finally, the use of rTMS should be systematically considered as an add-on treatment in combination with medication, physiotherapy, or psychotherapy, with the aim of improving or accelerating the efficacy of these therapeutic approaches.

This combined strategy, which is currently used in in depression, in combination with antidepressant drugs, and in stroke rehabilitation (with rehabilitation), will possibly boost up processes of cortical plasticity improving and stabilizing the therapeutic effects of rTMS.

CONCLUSIONS

The methodological improvements and the application of the rigid rules of clinical drug trials may hopefully help to reduce the large inter-individual variation in efficacy that currently makes the final clinical outcome rather modest, although the effects may be very pronounced and even long-lasting in individual patients.

AUTHOR CONTRIBUTIONS

CT, VR, and AlbC conception and idea of the paper. GC, AleC, and DM critical analysis of the literature. AQ conception and idea of the paper, data interpretation, overall supervision of the review. All authors discussed the results and contributed to the final manuscript.

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Pre-surgical Brain Mapping: To Rest or Not to Rest?

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Preoperative mapping of brain functions is the most common clinical application of functional MRI (fMRI) (1). The pre-surgical localization of eloquent areas has a positive impact for maximizing the extent of resection while reducing intra-operative mapping time (2) and improving patient outcome (3). In the pre-surgical setting, the typical fMRI approach employs conventional tasks which require patients to execute simple tasks in the scanner (4–8). This task-based fMRI (tb-fMRI) approach is well established and widely used in clinical routine, but has limitations: patients must be able to perform the tasks appropriately, implementation has a cost, and trained personnel is needed to select the proper task and instruct the patient.

One straightforward alternative to task-based fMRI is resting-state fMRI (rs-fMRI). This technique allows the study of spontaneous, low-frequency fluctuations that occur throughout the brain and has been widely used to characterize the healthy brain and neurological diseases. With rs-fMRI it is possible to identify a number of networks, or components, which are strongly functionally connected at rest and highly reproducible across subjects and sessions (9–11). Some of them show similar topographies to tb-fMRI networks, that is regions activated during cognitive functions, visual or sensory-motor tasks (12, 13). Measures of functional connectivity allow the study of brain functional reorganization and neuronal changes associated with brain disease (14). Alterations of rs-fMRI networks have been identified in many neurological and psychiatric disorders, even in absence of structural modifications, and in some studies have been shown to correlate with disease progression and severity (15).

Rs-fMRI has been applied also in the preoperative setting to overcome the limitations of task-based fMRI, including the need of active patient participation (16). rs-fMRI has the additional advantage that acquisition is brief (∼6–10 min) and can be easily managed by MRI technicians. Initial studies to validate the technique show good concordance with the gold standard intraoperative electro-cortical stimulation (ECS) (17–20). The most commonly identified networks for pre-surgical planning are the sensori-motor component, encompassing pre- and post-central cortex with the supplementary motor area, and the language network including Broca and Wernicke's areas, often corresponding to the fronto-parietal network (21–23).

OPEN ISSUES FOR PRESURGICAL rs-fMRI

Despite promising results, there remain open issues for the use of rs-fMRI in the preoperative setting. The methodology is the most critical issue: with rs-fMRI the acquisition is easy, but analyses are complex and have not been standardized yet. Multiple analysis techniques are available and, according to the method, different results can be obtained from the same dataset (11, 24, 25). Identification of the network is a critical step and can occur: (i) automatically or semi-automatically with Independent Component Analysis (ICA), a data-driven method commonly used: a network among a set of components is selected either visually (26) or through a spatial matching with respect to network templates (17, 27–29); (ii) manually, with a seed-based approach, where pre-defined region-of-interest (ROI)s or seeds are selected based on

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a-priori hypothesis; (iii) with alternative methods, such as machine learning approaches (18, 30, 31), cortical parcellating approach (19) or graph analyses (15).

Networks are not all as easily identifiable. The sensori-motor component is one of the lower-level networks with a known functional correlate (i.e., it corresponds to the sensori-motor system) which is robust and easy to identify at individual level (32). On the other hand, the identification of the language component is more challenging. Language is a "higher-order" function with substantial variability across subjects and several cognitive functions such as comprehension, production and perception (if a word is presented visually) are involved in language. For this reason, it remains to be determined whether a single language network can be observed. In addition, when language is mapped with ICA, the network showing the highest spatial overlap with the task-based map or known language areas is used (26, 28, 33, 34), while when it is mapped with the seed-based analysis, language ROIs of the left or right hemisphere or electrically stimulated points as seeds are used (17, 35, 36).

Language lateralization is also important in preoperative mapping, as surgical interventions in the dominant hemisphere are at higher risk of post-operative language deficits than interventions in the non-dominant hemisphere (37). rs-fMRI has been shown to provide results that are generally concordant with the Wada test for language dominance, similarly to tb-fMRI (33, 38). However, the degree of language lateralization measured with rs-fMRI varies considerably, depending on regions and methods used (35, 36, 39, 40). More studies assessing rs-fMRI lateralization with clinical, neuropsychological and post-surgical outcome evaluations are needed in order to determine which method (ICA or seed) provides the most reliable results (34, 41). At present, it is still unclear which method of analysis produces the most reliable results for mapping eloquent areas.

The second issue concerns the differences between rs-fMRI and tb-fMRI. The rs-fMRI map is different from the tb-fMRI one, as the two techniques are intrinsically different. As recently reported by Derks et al. (42), the two imaging methods measure different aspects of brain function. Tb-fMRI is related to the performance of a task or administration of a stimulus, and the resulting maps represent the brain areas involved. By contrast, rs-fMRI refers to brain's intrinsic activity, the degree of communication between areas and the resulting maps represent networks of synchronous BOLD activity (43). This may explain why the degree of overlap between the two techniques is not complete (30, 44, 45). In particular, the motor network observed at rest often covers a larger portion of the motor cortex compared to the more focal region identified with tb-fMRI (30, 44). Understanding whether a region is necessary for a function might be more difficult with rs-fMRI; in practice, future studies comparing rs-fMRI with ECS data will evaluate the accuracy of rs-fMRI results, using sensitivity and specificity measures (17–20). In other cases, the rs-fMRI motor network covers a smaller portion of the motor cortex when, for instance, a high number of components is selected with ICA, and the foot or mouth motor areas can be difficult to identify with ICA (44, 46).

An example of motor and language mapping performed with rs-fMRI and ECS is illustrated in **Figure 1**. Motor mapping performed with rs-fMRI through a motor seed (placed in the healthy Rolandic cortex), provided a larger portion of sensori-motor areas compared to the finger tapping map (in green). In the same patient, ICA showed a different localization of the hand motor area compared to tb-fMRI and seed-based analysis. Nevertheless, the stimulation site which elicited motor responses was included in both rs-fMRI and tb-fMRI activations (patient 12 in Rosazza et al. (44)). In another patient, language mapping performed with rs-fMRI data through a language seed (placed in the left inferior frontal gyrus) and ICA, localized, to some extent, different language areas, even if the stimulation site was included in the activation pattern (patient 6 in Zacà et al. (47)).

Among the factors that contribute to different rs-fMRI patterns, there is the placement of seeds when using the seed-based analysis, as seed size and location can bias the network, especially in lesioned brains. By contrast, when using ICA, networks can be combined or split, according to the small or big number of components used. In general, although ICA has ambiguity in choosing the number of components and identifying the components of interest, it is more explorative and less subjective than seed-based approach. Therefore, it may work better in patient populations, especially when large lesions may prevent the identification of reliable seeds in the eloquent cortex. In comparison with rs-fMRI, also tb-fMRI has some drawbacks, including: the choice of tasks used in particular for language mapping, the use of active vs. control conditions where it can be difficult to match the cognitive demands of the conditions and finally the use of two tasks that can be more time consuming than a single rs-fMRI sequence.

The third issue concerns the interpretation of rs-fMRI results. The pattern of correlated activity observed with rs-fMRI is not easy to interpret. With tb-fMRI, a map is associated to a behavior or cognitive function and this makes the results quite clear: in language mapping, for example, anterior regions are expected to be activated for production and so are posterior regions for comprehension; in addition, a differential involvement of anterior vs. posterior temporal regions is typically observed depending on the task (48, 49).

With rs-fMRI it is more difficult to understand the functional specificity of the map and interpret the results. The interpretation is even more difficult when rs-fMRI maps do not include expected areas, such as the paracentral lobule of the sensori-motor network. Most importantly, correlations of rs-fMRI pattern with cognitive or behavioral data have been established only occasionally. For instance, the rs-fMRI study by Otten et al. (50) has shown that in patients with brain tumors, motor weakness was associated with reduced connectivity of the sensori-motor network between inter-hemispheric motor areas. Further studies correlating measures of functional connectivity with clinical and cognitive data are necessary to understand the value of rs-fMRI in the clinical setting.

FUTURE DEVELOPMENT OF rs-fMRI

Rs-fMRI has begun to make clinically meaningful contribution to the localization of eloquent areas. To make rs-fMRI standard

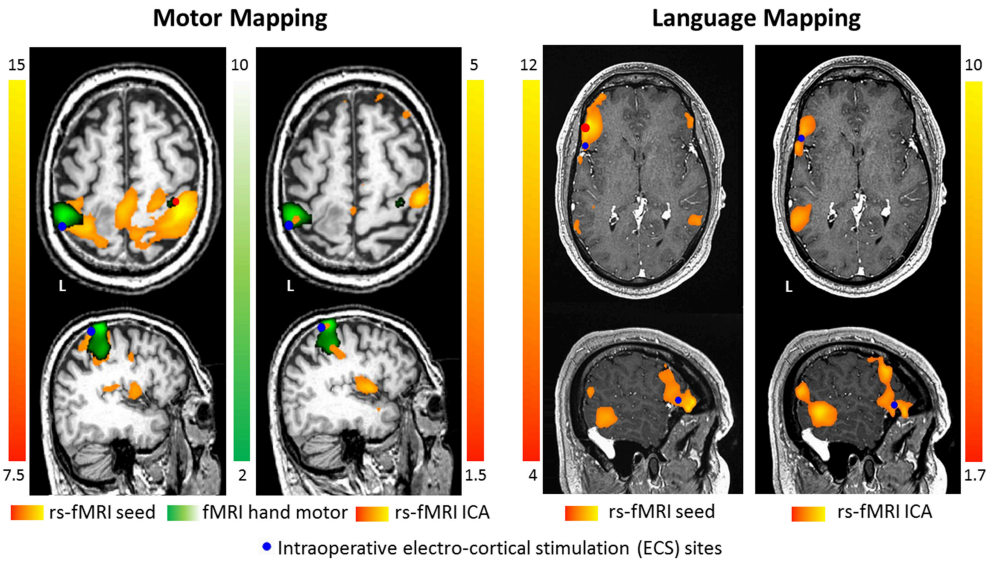

FIGURE 1 | Motor mapping was performed in a surgical patient using rs-fMRI with motor seed (in red, placed in the healthy Rolandic cortex), rs-fMRI ICA, a finger tapping motor task and ECS data (patient 12 in Rosazza et al. (44)). Results showed that seed-based analysis, ICA and tb-fMRI, provide comparable but not equivalent results; nevertheless, the stimulation site (in blue) was included in each activation pattern. In another surgical patient language mapping was performed using rs-fMRI with language seed (in red, placed in the left inferior frontal gyrus), rs-fMRI ICA and ECS data (patient 6 in Zacà et al. (47)). Results showed that seed-based analysis and ICA provided similar but not equivalent results; however, stimulation site (in blue) was included in the pattern of correlated activity.

of care for pre-surgical mapping, further developments in the methodological and theoretical setting are needed. From a methodological point of view, analyses must be reliable, quick and easily applicable in the preoperative routine for single patients. Initial steps have been made in this direction (51–53); however the procedure of analysis must be standardized, validated with ECS data and replicated in large population studies.

We can hypothesize that for motor mapping, the manual approach with the seed-based analysis will be better suited to localize the area of interest (foot, hand or mouth) with respect to the lesion. In fact, the seed-based analysis offers the flexibility necessary to explore the functional connectivity from different ROIs, even if it is more sensitive to the type of preprocessing performed. However, when the identification of motor seeds is confounded by pathology, ICA could provide a valid alternative approach (30, 53). In contrast, for language mapping, a semi-automatic approach with ICA could be better suited to assess lateralization and also localize language. ICA could be repeated setting a different number of components (e.g., 10, 20, 30..) and selecting the network that best matches a pre-defined template. This approach has been shown to provide reliable results because it takes into account both the variability of optimal number of components and localization of language areas across patients (52). When possible, the two methods of analysis should be applied jointly to obtain an independent confirmation of findings, similarly to the tb-fMRI procedure where two or more tasks are typically administered to map a function.

Considering the current limitations, we believe that, at present, tb-fMRI represents the first paradigm to choose for preoperative mapping of brain functions. Tb-fMRI is more robust with respect to noise, different tasks can be employed to map specific areas, analyses are quick, widely applied in the clinical routine, and maps are easy to interpret. When tb-fMRI cannot be used for clinical or logistic reasons, for instance because patients cannot perform the task or the stimulus delivery device is not available, then rs-fMRI will be used for the same purpose.

However, looking forward, we believe that, in the future, conceptual and methodological advances in neuroimaging techniques will allow a broader application of rs-fMRI functional connectivity in different neurological disease, including surgical practice. We are in fact shifting from a localizationist vision to a network-centric perspective, according to which the brain is organized into hierarchical, integrated, and interconnected large-scale networks, and neurological diseases are described as neuronal circuits dysfunctions (54–56). Network modeling of neuro-pathological conditions will be widely performed through connectivity analyses within and between networks, and results will be easily visualized to make rapid clinical decisions (15, 57). This is going to be accompanied by important theoretical advancements in clinical neuroscience: the functional relevance of rs-fMRI measures and their clinical correlates will be elucidated, together with a clearer definition of the areas of networks indicating eloquent cortex. rs-fMRI is going to be used also to localize glioma-related alterations and delineate the degree of tumor infiltration (14). From longitudinal studies it is going to

be possible to understand network changes occurring throughout surgery and this will allow the development of personalized treatments (58). In this context, we can imagine that rs-fMRI will be used in the preoperative setting not only to map eloquent areas but also to get information about changes on neuronal circuits caused by lesions, and eventually it will provide the basis for a multi-network assessment for diagnosis, prognosis and treatment of single patients.

In conclusion, methodological and theoretical limitations currently prevent a routine use of rs-fMRI in the pre-surgical setting. However, there is a wider potential for this technique that it is likely to be realized in the future also for preoperative mapping.

AUTHOR CONTRIBUTIONS

CR and MB suggested the idea of the work. CR and DZ drafted the manuscript. CR, DZ, and MB revised the manuscript. All authors have read and approved the manuscript in its final form.

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SYSTEMATIC REVIEW
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Anticonvulsants for Psychiatric Disorders in Children and Adolescents: A Systematic Review of Their Efficacy

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Aim: Anticonvulsant medications are frequently used in clinical practice to treat psychiatric disorders in children and adolescents, but the evidence for their efficacy is uncertain. We conducted a systematic review of published randomized controlled trials (RCT) that assessed the psychiatric benefit of anticonvulsants in patients under 18 years of age.

Method: The Medline, Scopus, Web of Science, and ClinicalTrials.gov databases were systematically searched for peer-reviewed primary publications of RCTs with a minimum of 10 patients per treatment arm through December 2017.

Results: Out of 355 identified non-duplicative publications, 24 met the inclusion criteria. Most RCTs were to treat bipolar disorder ($n = 12$) or manage recurrent aggression ($n = 9$). Few ($n = 3$) had both a multisite design and adequate statistical power. Valproate was the most frequently studied anticonvulsant ($n = 15$). Out of three placebo-controlled RCTs of valproate in bipolar disorder, none showed efficacy. In four RCTs, valproate was inferior to the antipsychotic risperidone. In several small, single-site RCTs, valproate and sulthiame were better than placebo for the management of recurrent aggression.

Conclusions: Currently available RCTs do not support the efficacy of anticonvulsants as mood stabilizers in children. There is some preliminary evidence from small RCTs of the efficacy of some anticonvulsants in the control of aggression and behavioral dyscontrol in conduct disorder, autism, and intellectual disability.

Keywords: anticonvulsants, children, psychiatric, bipolar, aggression, clinical trial

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INTRODUCTION

Anticonvulsant medications have been used for decades in the treatment of psychiatric disorders. It is postulated that the biochemical mechanisms underlying their anti-seizure activity can lead also to stabilization of mood and behavior (1). In adults, valproate, carbamazepine, and lamotrigine have demonstrated efficacy as mood stabilizers in acute mania and/or as maintenance treatment of bipolar disorder to prevent recurrence (2–5). Oxcarbazepine and topiramate are also used, but without clear-cut evidence of efficacy (6–8). In addition, some anticonvulsants have anti-aggressive properties, and carbamazepine, oxcarbazepine, and phenytoin have been found to be effective in the management of recurrent impulsive aggression (9).

In children (here intended as individuals under 18 years of age), anticonvulsants are frequently used to stabilize mood and behavior, usually in the context of bipolar disorder or other disorders that are accompanied by recurrent aggression, self-injury, or severe temper dysregulation, such as intellectual disability, autism spectrum disorder, conduct disorder, and attention deficit-hyperactivity disorder (ADHD) (10). In fact, anticonvulsants have been among the most commonly used pharmacological agents in pediatric bipolar disorder (11).

No anticonvulsant currently carries regulatory approval for pediatric use for the treatment of bipolar disorder or other psychiatric indications. Thus, anticonvulsants are used "off label" in children. Uncontrolled investigations have been indeed suggestive of efficacy (12). Uncontrolled studies, however, cannot constitute evidence of efficacy, especially in psychiatric conditions, such as mood disorders, that are characterized by high rates of spontaneous improvement and placebo effect. Only randomized controlled trials (RCTs) can demonstrate efficacy.

In order to evaluate the evidence for the efficacy of anticonvulsants in the treatment of psychiatric disorders in children, we conducted a systematic review of RCTs. The main aim was to identify which anticonvulsants, if any, have proven efficacy in the treatment of psychiatric disorders in children. According to evidence-based medicine standards, efficacy would be proven if supported by at least two independent RCTs.

METHODS

The standard methodology of systematic reviews was applied (13).

Selection Criteria

We searched for English language, peer-reviewed publications that were the primary reports of RCTs testing the efficacy of anticonvulsants in the treatment of psychiatric disorders in children. Included were all anticonvulsants with proven anticonvulsant effects and currently approved for the treatment of epilepsy. The psychiatric conditions included: mood disorders (depression and bipolar disorder), conduct disorder, recurrent aggression, ADHD, anxiety, autism spectrum disorder, eating disorders, and tic disorders. Excluded was the use of anticonvulsants for migraine, headache, neuropathy, or pain management. Excluded were also RCTs in which the anticonvulsant was not the independent variable being tested for efficacy. A minimum sample size of 10 children randomized to each treatment group was required for inclusion. RCTs that enrolled adults, in addition to children, were included only if the study sample had a preponderance of subjects under 18 years of age.

Search Mechanism

The Medline, Web of Science, and Scopus databases were systematically searched for English language publications through December 2017. The search inputs were: "anticonvulsant and children (age 0–17 years) and psychiatric disorder or bipolar disorder or mania or depression or anxiety or aggression or autism or conduct disorder or ADHD or Tourette or eating disorder," repeated for specific anticonvulsant medication (i.e., valproate, carbamazepine, oxcarbazepine, lamotrigine, phenytoin, topiramate, gabapentin, pregabalin, levetiracetam, clonazepam, clobazam, perampanel). All searches used *clinical trial* as a filter. In addition, the ClinicalTrial.gov database was similarly searched for clinical trials of anticonvulsants in children for bipolar disorder, anxiety, ADHD, and autism.

Review and Selection Process

After removal of duplicates, the publication titles and abstracts were visually inspected and reviewed independently based on the selection criteria by two experts (CD and BV). Disagreements were discussed and resolved by consensus in order to arrive at an agreed upon list of RCT publications.

Analysis

A qualitative analysis of the selected studies was independently conducted, based on the study reported characteristics and outcomes. The characteristics, quality, and limitations of each selected RCT were assessed based on the published report. Disagreements between raters were resolved by discussion and consensus. When additional information was needed, the corresponding author of the report was contacted in an attempt to acquire additional information. In assessing quality of each study, the presence of the following seven elements was examined: (1) double-blind design; (2) description of randomization and masking methods to minimize assessment biases; (3) multiple clinical sites (more than one); (4) sample size of at least 40 children randomized to each treatment group to provide statistical power to detect medium treatment effect sizes; (5) sufficient exposure to treatment with respect to dosage (i.e., dose in the known therapeutic range for anticonvulsant action, supported by plasma levels when available) and duration of treatment (at least 3 weeks for acute efficacy and at least 12 weeks for efficacy of maintenance treatment); (6) acceptable sample retention (<25% of the randomized sample lost to follow-up); and (7) intent-to-treat analyses.

RESULTS

The initial search yielded a total of 351 non-duplicate publications; an additional 4 publications were identified through manual reference review or other sources. Of these 355 publications, 331 were excluded as not meeting the pre-specified selection criteria, being 9 not in English, 11 not on anticonvulsants, 136 not for psychiatric use, 162 not RCT, 2 with too small of a sample size, and 11 secondary analyses of RCT (see **Figure 1**).

A total of 24 publications, each constituting the primary report of a RCT of anticonvulsant efficacy in psychiatric disorder in children were identified (see **Tables 1**, **2**). Half of these RCTs were in the treatment of bipolar disorder, including acute control of manic/mixed episodes and chronic maintenance to prevent recurrence (see **Table 3**). The other most common psychiatric use was for the control of recurrent impulsive aggression, mainly in the context of a neurodevelopmental disorder, such as autism and/or intellectual disability ($n = 9$).

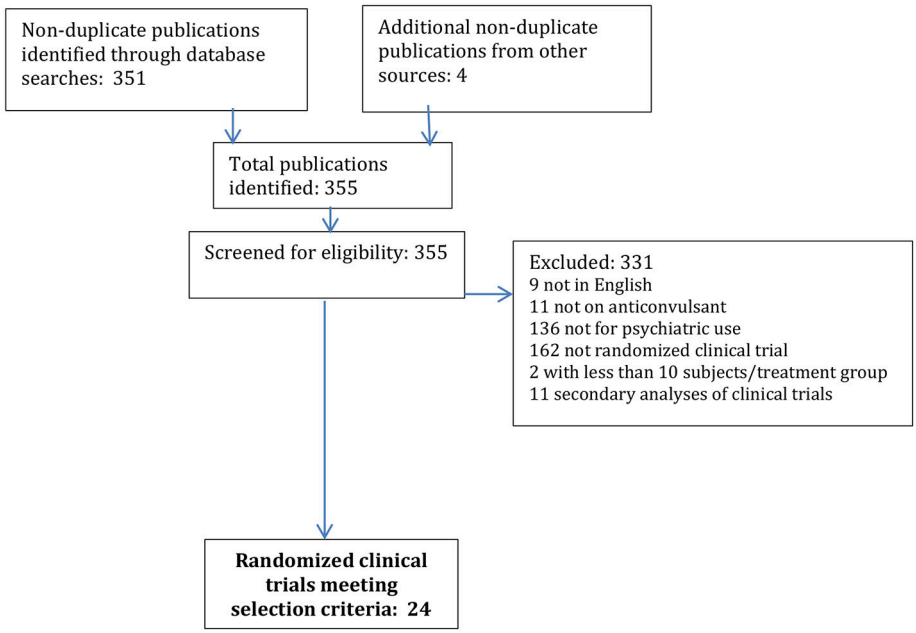

FIGURE 1 | Review flowchart.

The RCTs were conducted in double-blind conditions, except for four, which, however, employed masking methods (i.e., blinded raters) to limit ascertainment biases. Most were placebo-controlled, while four were comparative effectiveness RCTs of different active medications without a placebo control. The age of the RCT samples was mainly between 5 and 17 years. Only two RCTs included preschoolers as young as 3 years of age (21, 32).

Treatment exposure, with respect to adequate dosage and sufficient duration, as well as retention and statistical analyses, were considered to be satisfactory, but only five RCT involved more than one site and only five had a sample size of at least 40 subjects per treatment group (see Supplementary Table 1). Of the 24 RCTs, 13 (54%) did not find a statistically significant difference (**Tables 1**, **2**).

Only two RCTs were deemed to have met all the specified seven quality elements (17, 24), and, in particular, to have adequate sample size. Neither of these studies found the anticonvulsant medication to be better than placebo.

Most of the RCTs evaluated valproate ($n = 15$), while three tested carbamazepine (one of these studies included also valproate). The remaining anticonvulsants (oxcarbazepine, lamotrigine, levetiracetam, clonazepam, topiramate, and sulthiame) had only one or two RCTs each. Valproate was tested as a mood stabilizer in bipolar disorder in 10 RCTs and in the prevention of recurrent aggression in five RCTs. In bipolar disorder, none of the three placebo-controlled RCTs showed efficacy (16, 17, 21). Four RCTs showed superiority of the antipsychotic risperidone over valproate (18–21). No difference was detected between valproate and lithium (14), carbamazepine (23), or quetiapine (15). Finally, one RCT conducted to test the antimanic effects of topiramate in hospitalized youths, using valproate as a comparison group, found valproate to be superior to topiramate (22).

As anti-aggressive agent, valproate showed no difference from placebo in one RCT (29) and was better than placebo in three small RCTs, one in children with autism spectrum disorder (30) and two in children with conduct disorder or ADHD (26, 28). One RCT that compared high with low dose of valproate found superiority of the higher dose (27).

Four RCTs tested carbamazepine or oxcarbazepine in the treatment of mania, aggression, or ADHD. Two of these RCTs found no difference from placebo (24, 31). Another RCT found carbamazepine inferior to clonidine in ADHD (32), and in the third one there was no difference vs. valproate (23).

No evidence of efficacy emerged for lamotrigine, levetiracetam, and clonazepam. In two RCTs that were conducted more than 40 years ago in institutionalized, severely impaired subjects with intellectual disability, including both youths and adults, sulthiame, was better than placebo for controlling aggression and hyperactivity (36, 37).

Conclusions

This systematic review identified mostly small controlled studies with important methodological limitations and heterogeneity with respect to type of medication and clinical target. No evidence emerged for the efficacy of anticonvulsants in children with bipolar disorder. There is limited evidence for the efficacy of valproate and sulthiame for the management of aggressive behavior. For sulthiame studies were conducted in samples that included adult patients and the specific efficacy in children cannot be estimated.

TABLE 1 | Randomized controlled clinical trials assessing the efficacy of anticonvulsant medications in the treatment of children (under 18 years of age) with bipolar disorder ($n = 12$)[a].

Medications	Psychiatric disorder	Target	Design	Sample	Outcome measures	Dosage/Serum level	Results[b]	References
Valproate	Bipolar I or II disorder	Maintenance of mood stability	Discontinuation design: after stabilization on Li plus DVPX, randomization to Li or DVPX monotherapy for 18 months	$n = 60$ age:5–17 y	Time to relapse (mood instability)	DVPX: 20 mg/kg/d. Serum valproic acid level: 75 mcg/mL (mean)	No difference between Li and DVPX	Findling et al. (14)
Valproate	Bipolar I acute mania or mixed episode	Mania, irritability	Randomization to DVPX or quetiapine for 4 weeks	$n = 50$ age:12–18 y	YMRS	Serum valproic acid level: 80–120 mcg/mL	No difference between DVPX and quetiapine on YMRS scores. More rapid symptom decrease with quetiapine	DelBello et al. (15)
Valproate	Bipolar NOS, cyclothymia	Maintenance of mood stability	Randomization to DVPX or placebo for up to 5 years	$n = 56$ age:5–17 y	Time to drug discontinuation	Up to 15 mg/kg/d (maximum: 1250 mg/d)	No difference between DVPX and placebo	Findling et al. (16)
Valproate	Bipolar I acute mania or mixed episode	Mania, irritability	Randomization to DVPX or placebo for 4 weeks	$n = 150$ age:10–17 y	YMRS CGI-I CGI-S	DVPX: 1,286 mg (mean). Serum valproic acid level: 80 mcg/mL (mean)	No difference between DVPX and placebo.	Wagner et al. (17)
Valproate	Bipolar I acute mania or mixed episode	Mania, irritability	Randomization to DVPX or risperidone for 6 weeks	$n = 66$ age: 8–18 y	YMRS	DVPX: up to serum valproic acid level of 60–120 mcg/mL	Risperidone superior to DVPX	Pavuluri et al. (18)
Valproate	Bipolar I acute mania or mixed episode	Mania, irritability	Randomization to DVPX, lithium or risperidone for 8 weeks. Open study, with blinded raters	$n = 290$ age: 6–15 y	CGI-I for bipolar symptoms	Serum valproic acid level: 113.6 mcg/mL (mean)	Risperidone superior to DVPX and lithium. No difference between DVPX and lithium	Geller et al. (19)
Valproate	Bipolar I acute mania or mixed episode	Mania, irritability	Randomization to switching to or adding DVPX, lithium or risperidone for 8 weeks. Open study, with blinded raters	$n = 154$ age: 6–15 y	CGI-I for bipolar symptoms	Up to valproic acid serum levels of 111–125 mcg/mL	Risperidone superior to DVPX and lithium	Walkup et al. (20)
Valproate	Bipolar I acute mania or mixed episode	Mania, irritability	Randomization to valproate, risperidone, or placebo for 6 weeks	$n = 46$ age: 3–7 y	YMRS	Up to valproic acid serum levels of 80–100 mcg/mL	No difference between valproate and placebo. Risperidone superior to placebo.	Kowatch et al. (21)
Valproate, Topiramate	Bipolar I acute mania	Mania	Randomization to DVPX or topiramate for 8 weeks	$n = 142$ age: 12–18 y	YMRS	Valproate: up to 1,200 mg/d	Valproate superior to topiramate	Hebrani et al. (22)
Valproate, Carbamazepine	Bipolar I or II disorder, mixed or manic episode	Mania, irritability	Randomization to DVPX, carbamazepine or, lithium. Open study with blinded raters	$n = 42$ age: 11 y (mean)	YMRS	Up to serum valproic acid level of 85–110 mcg/mL and carbamazepine level of 7–10 mcg/mL	No difference between treatment groups	Kowatch et al. (23)
Oxcarbazepine	Bipolar I acute mania or mixed episode	Mania, irritability	Randomization to oxcarbazepine or placebo for 7 weeks	$n = 116$ age:7–18 y	YMRS	900–2,400 mg/d (mean 1,515)	No difference between oxcarbazepine and placebo	Wagner et al. (24)

(Continued)

TABLE 1 | Continued

Medications	Psychiatric disorder	Target	Design	Sample	Outcome measures	Dosage/Serum level	Results[b]	References
Lamotrigine	Bipolar I disorder	Maintenance of mood stability	Discontination study: after initial stabilization with other mood stabilizer plus lamotrigine, randomization to continuing lamotrigine or switching to placebo, for 36 weeks	$n = 60$ age:10–17 y	Time to occurrence of a bipolar event	Up to 240 mg/d	No difference between lamotrigine and placebo (secondary analysis: lamotrigine superior to placebo for 13–17 year old subgroup)	Findling et al. (25)

[a] *Double-blind masking unless otherwise specified.*
[b] *Statistically significant differences at $p \leq 0.05$.*
Bipolar NOS, bipolar disorder not otherwise specified (Diagnostic and Statistical Manual - IV edition); CGI-I, Clinical Global Impression-Improvement Scale; CGI-S, Clinical Global Impression-Severity Scale; d, day; DVPX, divalproex (formulation of valproate); Li, lithium; y, year; YMRS, Young Mania Rating Scale.

DISCUSSION

To evaluate the evidence for efficacy of anticonvulsant medications in psychiatric disorders of childhood, we conducted a systematic qualitative review of relevant published RCTs. We restricted the search to RCTs because uncontrolled studies cannot provide evidence of treatment effects given the variable placebo-response in psychiatric conditions.

Twenty-four RCTs met the pre-specified selection criteria (**Tables 1**, **2**). The medication dosage and the duration of treatment were generally appropriate, and many studies measured medication serum levels. Most of these RCTs, however, had important methodological limitations, especially a small sample size (<40 per treatment group), and therefore inadequate statistical power to detect medium effect sizes (see Supplementary Table 1).

A sample size of 40 subjects per treatment group will provide 80% statistical power to detect a between-group effect size usually considered in the medium range (e.g., a Cohen's $d = 0.6$) as statistically significant at a $p \leq 0.05$ (38). The small sample size of the large majority of these RCTs strongly limits their capacity to identify statistically significant treatment differences. In fact, out of 24 RCTs, 13 (54%) did not find a statistically significant difference between the treatment groups.

A considerable number of RCTs was conducted on valproate ($n = 15$), three of which had adequate sample size, while few studies were devoted to other anticonvulsants. In the treatment of children with bipolar disorder, valproate showed no evidence of superiority over placebo, and was actually inferior to risperidone based on four RCT (**Table 1**).

Two RCTs compared valproate to lithium or quetiapine, respectively, in bipolar disorder, and found no difference between treatment groups (14, 15). Considering the small sample size of these RCTs and the lack of a placebo condition, the lack of difference cannot be interpreted as evidence of efficacy. Another, single-site, RCT compared topiramate to valproate in hospitalized, acutely manic youths (22). This study, which had been designed to test the efficacy of topiramate, found valproate to be superior to topiramate. The report, however, lacks an adequate description of the masking methods, and the especially large effect size is surprising and possibly due to the specific context of the hospital where the study was conducted.

These data on anticonvulsants in child bipolar disorder appear to be at odds with the evidence for efficacy of valproate, carbamazepine, and lamotrigine in adults with bipolar disorder. In adults, valproate and carbamazepine are superior to placebo in acute mania (2, 3), although less effective than antipsychotics (3), and lamotrigine is superior to placebo in bipolar depression (4). More limited evidence supports also the efficacy of valproate as maintenance treatment in adult bipolar disorder (5). The discrepancy between adults and children is suggestive of developmental differences in the psychopathology of the mood dysregulation. It should also be pointed out that there are several psychiatric medications, such as antidepressants and benzodiazepines, whose efficacy has been shown in adults but not in children (39).

TABLE 2 | Randomized controlled clinical trials assessing the efficacy of anticonvulsant medications in the treatment of children (under 18 years of age) with psychiatric disorders other than bipolar disorder ($n = 12$)[a].

Medications	Psychiatric disorder	Target	Design	Sample	Outcome measures	Dosage/serum level	Results[b]	References
Valproate	ODD or CD	Explosive temper, mood lability, aggression	Randomization to DVPX or placebo for 6 weeks (phase 1) followed by cross-over to other treatment for 6 weeks (phase 2)	$n = 20$ age: 10–18 y	Modified Overt Aggression Scale	DVPX: 750-1,500 mg/d	DVPX superior to placebo in phase 1. No difference in phase 2	Donovan et al. (26)
Valproate	CD	Explosive temper, mood lability, aggression	Randomization to low or high dose of valproate for 7 weeks	$n = 71$ age: 16 y (mean)	CGI-S CGI-I	Low dose: up to 250 mg/d High dose: 500-1,500 mg/d	High dose of valproate superior to lower dose	Steiner et al. (27)
Valproate	ADHD with ODD or CD	Aggression	Initial treatment with stimulant monotherapy, followed by randomization to DVPX or placebo for 8 weeks	$n = 30$ age: 6–13 y	Retrospective-Modified Overt Aggression Scale	20 mg/kg/d. Valproic acid serum level: 68.1 mcg/mL (mean)	DVPX superior to placebo	Blader et al. (28)
Valproate	Autism spectrum disorder	Aggression	Randomization to valproate or placebo for 8 weeks	$n = 30$ age: 6–20 y	ABC CGI-I	Valproic acid serum level: 77.8 mcg/mL (mean)	No difference between valproate and placebo	Hellings et al. (29)
Valproate	Autism spectrum disorder	Irritability/ Aggression	Randomization to DVPX or placebo for 12 weeks	$n = 27$ age: 5–17 y	ABC CGI-I	DVPX: up to 1,000 mg/d	DVPX superior to placebo	Hollander et al. (30)
Carbamazepine	CD	Aggression	Randomization to carbamazepine or placebo for 6 weeks	$n = 22$ age: 5–12 y	Overt Aggression Scale, CGI, Children's Psychiatric Rating Scale	200–800 mg/d (mean 683) Serum carbamazepine levels: 5.0-9.1 mcg/mL	No difference between carbamazepine and placebo	Cueva et al. (31)
Carbamazepine	ADHD	ADHD symptoms	Randomization to carbamazepine or clonidine for 4 weeks	$n = 50$ age: 4–12 y	Vanderbilt ADHD Rating Scale	Unspecified	Clonidine superior to carbamazepine	Nair and Mahadevan, (32)
Levetiracetam	Tourette disorder	Tics	Within-subject, crossover with randomization to levetiracetam or placebo, sequentially, for 4 weeks each	$n = 22$ age: 8–16 y	Yale Global Tic Severity Scale	Up to 30 mg/kg/d	No difference between levetiracetam and placebo	Smith-Hicks et al. (33)
Levetiracetam	Autism spectrum disorder	Hyperactivity, impulsivity,aggression, and mood lability	Randomization to levetiracetam or placebo for 10 weeks	$n = 20$ age:5–17 y	CGI-I, ABC, Conners' Rating Scale-Revised	863 mg/d (mean)	No difference between levetiracetam and placebo	Wasserman et al. (34)
Clonazepam	Anxiety disorders	Decrease in anxiety symptoms	Within-subject, crossover with randomization to clonazepam or placebo, sequentially, each for 4 weeks	$n = 15$ age: 7–13 y	Children Manifest Anxiety Scale	Up to 2 mg/d	No difference between clonazepam and placebo	Graae et al. (35)
Sulthiame	Intellectual disability	Hyperactivity, aggression	Within-subject, crossover with randomization to sulthiame or placebo, sequentially, each for 6 weeks	$n = 42$ age:7–38 y (mean 17)	Behavior rating scale	Up to 600 mg/d	Sulthiame superior to placebo	Moffat et al. (36)
Sulthiame	Intellectual disability	Hyperactivity, aggression	Randomization to sulthiame or placebo for 14 weeks	$n = 34$ age: 6–24 y	Behavior rating scale	Up to 15 mg/kg/d	Sulthiame superior to placebo	Al-Kaisi and McGuire, (37)

[a] *Double-blind masking unless otherwise specified.*

[b] *Statistically significant differences at $p \leq 0.05$.*

ABC, Aberrant Behavior Checklist; ADHD, attention deficit/hyperactivity disorder; CD, conduct disorder; ODD, oppositional defiant disorder.

TABLE 3 | Primary target of the 24 randomized controlled clinical trials (RCTs) of anticonvulsant medications in psychiatric disorders in children (under 18 years of age).

	no of RCTs
Control of acute symptoms of mania and irritability in bipolar disorder	9
Prevention of recurrent explosive aggression	9
Prevention of recurrence of bipolar acute episode	3
Control of symptoms of ADHD	1
Control of tics in tourette disorder	1
Control of symptoms of anxiety	1

For the management of aggression and explosive temper, valproate showed efficacy in four small and single-site RCTs in children with conduct disorder, ADHD, or autism (26–28, 30). One of these, used valproate as add-on treatment to stimulant medication in ADHD (28). Another one was designed as a crossover trial, but could not complete the second segment of the study and analyzed only the first part (26). Even if none of these studies met all the methodological quality criteria, these data can be taken as tentative evidence of efficacy of valproate in controlling aggressive behavior. This is consistent with the results of a meta-analysis of anticonvulsants in the management of aggression in adults (9). These findings, however, should be confirmed by adequately powered, multisite RCTs.

Little evidence of efficacy in bipolar disorder emerged from the RCTs of other commonly used anticonvulsants. In particular, a statistically powered, multi-site, placebo-controlled trial of oxcarbazepine in bipolar disorder found no statistically significant difference (24). Lamotrigine, which is effective in bipolar depression in adults, did no better than placebo in a RCT that included children aged from 10 to 17 years, although secondary analyses found superiority in the 13- to 17-year-old subgroup (26). Sulthiame, an infrequently used anticonvulsant, was found better than placebo in 2 RCTs conducted more than 40 years ago for the control of aggression and hyperactivity in institutionalized patients, including both youths and adults, with severe intellectual disability (36, 37), but the implications of these data for current practice are unclear.

The limitations of this review are primarily related to the design characteristics of the studies and especially to the small sample size of most of them. The studies are also rather heterogeneous with respect to both the type of anticonvulsant being tested and the clinical indication being targeted. Another limitation is the relatively wide range of patient age in most studies, some of which included also adults.

Based on the current data, the psychiatric use of anticonvulsants in children cannot be supported according to evidence-based standards. Their potential benefit valproate and sulthiame for the management of recurrent aggression, however, cannot be discounted. The potential benefit of these medications must be in any case balanced against the risk for adverse effects, including psychiatric ones (40).

In conclusion, the efficacy of anticonvulsants as mood stabilizers in children with bipolar disorder remains unproven. There is limited evidence that some anticonvulsants may decrease aggressive behavior and explosive temper, especially in patients with neurodevelopmental disorders and intellectual disability. Because this evidence comes mainly from small studies, it might be informative to conduct more precisely designed and adequately powered RCT targeting recurrent and impulsive aggression in children with neurodevelopmental disorders.

AUTHOR CONTRIBUTIONS

BV and CD were responsible for review, data extraction, and evaluation. CC, RV, and MG contributed expert review and helped in the data interpretation and manuscript preparation.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2018.00270/full#supplementary-material

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MINI REVIEW
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Innovation in Systems of Care in Acute Phase of Ischemic Stroke. The Experience of the Catalan Stroke Programme

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Stroke, and mainly ischemic stroke, is the second cause of death and disability. To confront the huge burden of this disease, innovative stroke systems of care are mandatory. This requires the development of national stroke plans to offer the best treatment to all patients eligible for reperfusion therapies. Key elements for success include a high level of organization, close cooperation with emergency medical services for prehospital assessment, an understanding of stroke singularity, the development of preassessment tools, a high level of commitment of all stroke teams at Stroke Centres, the availability of a disease-specific registry, and local government involvement to establish stroke care as a priority. In this mini review, we discuss recent evidence concerning different aspects of stroke systems of care and describe the success of the Catalan Stroke Programme as an example of innovation. In Catalonia, reperfusion treatment rates have increased in recent years and currently are among the highest in Europe (17.3% overall, 14.3% for IVT, and 6% for EVT in 2016).

Keywords: stroke, systems of care, innovation, endovascular treatment of stroke, thrombolysis

INTRODUCTION

Stroke—mainly ischemic stroke—is the second cause of death and disability worldwide (1). The stroke burden has increased across the globe in both men and women of all ages throughout the past two decades (2). However, population awareness of early symptoms, the accuracy of current brain imaging tests, and the development of acute therapies are contributing to reduce this trend (3). However, success will depend mainly on the structure of the healthcare system and it is uncertain whether systems in different countries are prepared to deal with this huge burden. Therefore, innovation in stroke systems of care is mandatory to transform them and prepare them to confront this health challenge.

INNOVATING IN SYSTEMS OF ACUTE STROKE CARE. ORGANIZATION AND COOPERATION TO DELIVER MORE TREATMENT, MORE RAPIDLY

The natural history of ischaemic stroke has changed dramatically since the 1990s. The beginning of the Intravenous Thrombolysis (IVT) Era in the late 90s (4) and recent approval of endovascular therapy (EVT) (5), the demonstration of improved stroke outcomes with stroke unit care, and the benefits of implementing organized stroke systems of care have all contributed to reduce mortality and disability in patients with acute ischaemic stroke (AIS) (3, 6).

The proportion of patients treated has increased in recent years in high-income countries, mainly in comprehensive stroke centers (CSCs), where EVT is provided (7). The effectiveness of reperfusion therapies is highly time-dependent: The sooner the patient arrives to the hospital after symptoms onset, the better. Once in hospital, highly organized workflows are of utmost importance to achieve door-to-needle times in IVT and door-to-groin-puncture times in EVT. A set of effective strategies has been successfully implemented in hospitals to reduce these critical time delays, especially pre-notification of arrival by Emergency Medical Services (EMS), direct transfer to the radiology service for brain CT scan or direct alteplase administration in the scanner (8, 9).

The eligibility of patients with AIS for reperfusion therapies is evolving as new evidence is published. Partially dependent patients, otherwise excluded from IVT trials, might benefit from thrombolysis (10) and certain patients with unknown time of stroke onset, precluded from seminal EVT trials, have shown improved outcomes after EVT (11, 12). Therefore, the overall proportion of AIS patients who might benefit from reperfusion therapies is rising and changing inclusion criteria generate uncertainty about how many more are potentially eligible.

The capacity to increase the proportion of patients treated is related to the structure of the system of care. Current evidence shows that patients who require interhospital transfer for EVT achieve reperfusion between 109 and 120 min later than those directly transported to CSCs, and have a lower absolute probability of independent outcome (13–15). These data should prompt a rethinking of systems of stroke care at national and regional levels in order to improve the "hub-and-spoke" transfer networks. This form of medical transport optimization organizes traffic routes as a series of 'spokes' that connect outlying points to a central 'hub.' In acute care for stroke, the Hubs are CSCs with great expertise that concentrate a huge volume of procedures and are connected with centers having a lower level of expertise and smaller volume of procedures.

UNDERSTANDING THE SINGULARITY OF STROKE TO IMPROVE CARE

Management of AIS has taken lessons from acute myocardial infarction management; however, unlike heart attack, in most cases acute stroke leaves patients unable to speak and to alert EMS by themselves. Population campaigns are crucial to raise awareness about stroke symptoms and how to detect them. In addition, EMS technicians must be specifically trained to detect stroke, activate the stroke code and pre-notify arrival to the nearest hospital with proper treatment capabilities.

The possibility of diagnostic testing in the ambulance to inform hospital treatment is another huge difference between heart attack and stroke. The recent development of CT-equipped mobile stroke units is considered an important advancement. This approach is safe and feasible, has increased IVT rates, and achieved significantly shorter time-to-treatment compared to conventional care in the areas tested, mainly in Germany (16). However, a recent study found no significant difference between the proportion of patients with a modified Rankin Scale score of 1 or less who received this type of care compared with conventional care (17). Therefore, due to its high cost without clear long-term benefit, we can conclude that an efficient technology for prehospital diagnosis of stroke that can be easily implemented is lacking.

ORGANIZING TO ACHIEVE BETTER RESULTS. ORGANIZATION AND COOPERATION AT DIFFERENT LEVELS OF CARE

Once the stroke code is activated, where do we transfer the patient? Given the beneficial results of bridging therapy (IVT plus EVT) in patients with large vessel occlusion (LVO) (5), it seems clear that the demand for neurointervention in coming years will grow in line with increasing numbers of EVT-capable centers. Nonetheless, it is difficult to justify the establishment of EVT-capable centers in remote areas with low population density.

Therefore, a crucial question is how to define the best transfer network for AIS patients located in remote and distant areas. The drip-and-ship model, which takes the patient to the nearest stroke center, prioritizes the initial diagnostic workup and IVT. In this model, the identification of an LVO patient is followed by interhospital transfer to a CSC. Another model is direct transfer to a CSC, thus bypassing the Primary Stroke Centre (PSC), known as the mothership model. A recent study in Canada used conditional probability modeling to find an answer, testing different transportation options to identify the better modeled outcome in specific regions. The authors concluded that a drip-and-ship model is appropriate if the treatment in a PSC is delivered in less than 30 min and the patient is then transferred to a capable CSC (18). In Catalonia, the ongoing RACECAT trial (Direct Transfer to an Endovascular Centre Compared to Transfer to the Closest Stroke Centre in Acute Stroke Patients with Suspected Large Vessel Occlusion; NCT02795962) is expected to provide answers to important questions of logistics and increase the efficient delivery of treatments and the number of acute stroke patients that have access to them.

There is still room for network innovation. In remote areas with no access to stroke experts, a possible and feasible solution is TeleStroke Centres (TSC). Using videoconferencing

and image-sharing technology, stroke specialists from a CSC can examine patients at remote hospitals to help with diagnosis and recommend a plan of care.

Results from a third model, called trip-and-treat, have been recently published. This urban interhospital service delivery model consists of a shared mobile interventional stroke team that travels to PSCs in New York City to provide on-site interventional capability. The authors concluded that, in their area of reference, the trip-and-treat model had shorter time-to-treatment for EVT, compared with drip-and-ship, offering a valid alternative to current interhospital stroke transfers in urban environments (19).

PREHOSPITAL ASSESSMENT

Theoretically, the benefits of a primary transfer to a CSC would only apply to patients with LVO and may unnecessarily delay treatment in all others. Therefore, the predictive power of initial screening tools to identify patients with suspected LVO becomes of paramount importance. Various scoring systems have been developed to detect potential candidates to EVT. These scales must meet key criteria: rapid and simple to use, applicable to an unselected population with a suspected stroke, high interrater reliability, and high accuracy to avoid underdiagnosis (low false-negative rate; i.e., 1-sensitivity) and overdiagnosis that could overload the CSC (low false positive rate, i.e., 1-specificity). Finally, the scale must be validated and proven to improve patient outcomes (20). A recent observational study compared 13 validated prehospital scales and concluded that published cutoff scores to predict LVO in clinical settings were associated with high accuracy but also yielded a high false-negative rate (21).

Among them, one that showed high accuracy and a comparatively lower false-negative rate was the RACE scale. This scale is a simplification of the NIHSS scale, using only those items with a higher ability to predict the presence of LVO. The RACE scale evaluates 5 items: facial palsy, brachial paresis, crural paresis, oculocephalic deviation and aphasia/agnosia. Scores range from 0 to 9. A score > 4 allows the suspicion of a LVO with a sensitivity of 85% and specificity of 69% (22). The RACE scale was designed and validated in Catalonia with a prospective study that included 357 patients in 2011–2013. Therefore, in optimizing stroke systems of care, the prehospital assessment is a key factor to take into account.

THE STROKE CODE SYSTEM OF CATALONIA AS AN EXAMPLE

Catalonia has a population of 7.5 M and an organized and highly territorialized stroke system of care administered by the Stroke Programme, an organization created in 2004 by the Catalan Health Department. In 2006, the Stroke Programme implemented the Stroke Code System in 2006 to cover all the territory (23) (**Figure 1**). Current criteria for Stroke Code activation are clinical suspicion of acute stroke, less than 8 h from symptom onset (or unknown), and previous functional independence (Rankin 0–2) with no age limit (24, 25). Upon

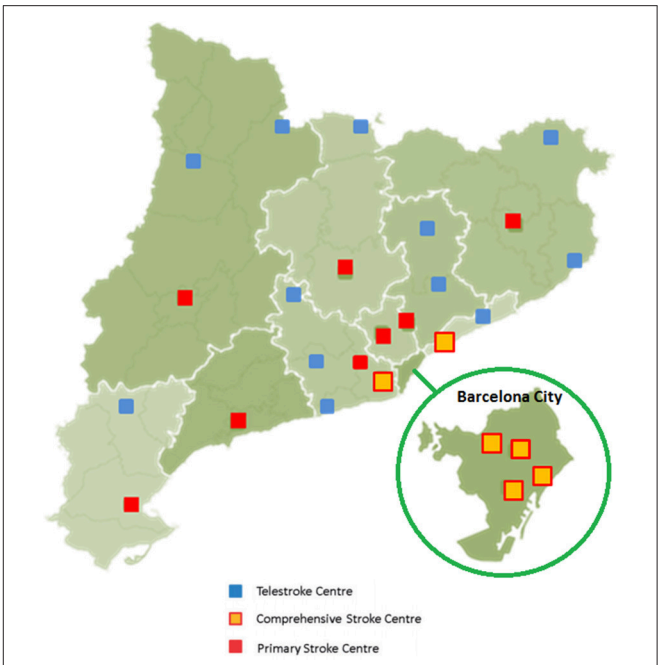

FIGURE 1 | Geographical distribution of the Stroke Centres in Catalonia.

Stroke Code activation, the EMS coordinates patient transport to the nearest Stroke Centre (SC) according to predefined pathways. After initial recognition of stroke symptoms, the destination SC is pre-alerted by the EMS. The Stroke Code can be activated directly from EMS upon identification of a stroke patient in the field (60% of all Stroke Code activations) or at the Emergency Department of any hospital when patients arrive at the hospital by their own means. In recent years, our network of acute hospitals that are active in the stroke code system has grown to include 26 centers: (1) Twelve TSCs with capacity to deliver IVT via tele-consultation with a vascular neurologist who covers all tele-consultations from the 7 TSCs in the outer metropolitan area of Barcelona; the remaining 5 TSCs are located in the Catalan provinces and are usually covered by the neurologist on call at the nearest provincial PSC, with the central on-call service acting as a backup. (2) Eight PSCs with capacity to deliver IVT and admit patients to a certified Stroke Unit. (3) Six EVT-capable centers or CSCs, all of them located in the inner metropolitan area of Barcelona.

According to the Stroke Code protocol, patients with a suspected acute stroke are transferred to the closest SC or TSC in order to prioritize urgent expert evaluation and rapid IVT if indicated. This strategy is extremely effective and safe, increasing IVT treatment and reducing the time from symptom onset to IVT initiation; however, this decentralized model is associated with delayed EVT initiation and lower rates of EVT, compared to areas where patients are directly transferred to a CSC (26).

Another important approach that has proved useful to improve the Stroke Code System in Catalonia has been the analysis of big data. The vast amount of clinical data

available constitutes a formidable resource for evaluation and research. Every day a large amount of stroke data (mostly unstructured or semi-structured) is generated in a great variety of sources that should be rapidly analyzed. One of these sources, population health registries, provides essential tools to obtain epidemiological data from patients with certain diseases, monitor therapies, and run audits of health services. This information is useful in the decision-making process when planning health policies in a given territory. Catalonia has a government-mandated, population-based registry (SONIIA) with external monitoring of data completeness, which assesses quality of reperfusion therapies delivered to ischemic stroke patients since 2011. Region-wide reperfusion treatment rates in Catalonia are among the highest in Europe (17.3% overall, 14.3 for IVT, and 6% for EVT in 2016, according to data from the Catalan Stroke Programme. **Figure 2** shows the temporal trends of population reperfusion therapies since 2005. The increased number of reperfusion treatments is likely a result of improved organization of stroke care, as well as the less restrictive criteria used for eligibility to treatments, which have changed through the years.

A decrease in mean door-to-needle and door-to-groin-puncture times has also been observed over time, being currently 38 and 75 min, respectively (taking into account all types of centers).

To achieve all these goals, a high level of commitment of all SC teams is necessary. Due to the different levels of complexity in the SCs in Catalonia, stroke patients are treated by multidisciplinary teams of healthcare professionals. According to data from a 2017 survey by the Catalan Stroke Programme, neurologists with expertise in cerebrovascular diseases, neuroradiologists, neurosurgeons, vascular surgeons, rehabilitation specialists (including physiotherapists) and stroke unit nurses are the core of the stroke teams in Catalan CSCs. The eight PSCs have a lower number of specialists and the disciplines involved are more diverse. Finally, in the 12 telestroke centers, the main healthcare professionals involved in stroke care are emergency physicians (internists). We consider that CSCs have homogeneous and well-balanced specialist teams and that less variability should exist in the PSCs, with stroke teams including neurologists, neuroradiologists, rehabilitation specialists (including physiotherapists) and stroke nurses.

We would emphasize that population campaigns are crucial. In Catalonia, the RAPID campaign (similar to Act FAST in the US) was launched in 2008 to increase the general public's knowledge about how to detect a stroke.

The RACE scale was incorporated into the Stroke Code protocol in Catalonia in September 2014, after an online training program for EMS technicians and other EMS professionals. Currently, the RACE scale is evaluated by the EMS team, registered on the EMS database, and delivered to the receptor Stroke Centre in >85% ($n = 5{,}073$) of the Stroke Code activations (2016 data, Stroke Programme).

Based on a well-established stroke network of acute hospitals that work in close collaboration with the EMS and the existence of an exhaustive, population-based registry validated for research quality, the Stroke Code system of Catalonia offers the potential for innovative studies, such as the RACECAT trial.

ENSURING UNIVERSAL ACCESS TO OPTIMAL TREATMENT. THE BIG CHALLENGE: EQUITY

Now that reperfusion therapies, specifically interventionism, have been shown to be beneficial in AIS patients with proximal LVO, stroke systems of care should be reorganized to provide acute treatment, including mechanical thrombectomy, in a timely and equitable manner. Recent studies, including one performed in Catalonia, have demonstrated that access to EVT from remote areas is limited in high-income countries (26) and geographic disparities in IVT use are increasing, showing a rural-urban inequality trend (27). In a rural area of North Carolina, researchers showed that re-organization of the stroke system of care (in that case, to pursuit official certification of the hospital)

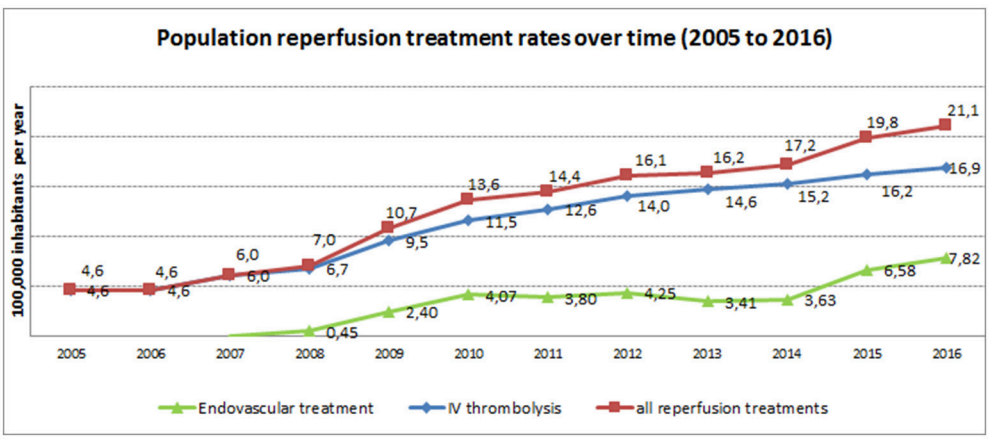

FIGURE 2 | Temporal trends of population reperfusion therapies rates (2005–2016).

allowed patients to receive evaluation and treatment in a timely and efficient manner close to home (28). A recent systematic review aimed to determine the quality of existing stroke-care services in low- and middle-income countries and described great variability, with very low rates of reperfusion therapies (and mainly IVT) provided in large part by the private sector (29).

Though differences in every country must be examined independently, there is still room for improvement in stroke care organization, using strategies that have been proven to be economic, feasible and reproducible, such as EMS training in pre-hospital assessment and telestroke implementation in remote areas (30). Other initiatives-such as education of the general population (i.e., public health campaigns), involvement of public-sector healthcare personnel, hospital preparedness, and legislative and economic factors-are key to success in improving access to best-practice stroke care (31).

CONCLUSIONS

Innovation in stroke systems of care is a key factor to achieve the main aim in stroke care: to build a national stroke plan capable of offering the best possible treatment to all patients eligible for reperfusion therapies. Necessary elements include a high level of organization, close cooperation with EMS (prehospital assessment), strong commitment of all stroke physicians at Stroke Centres, the availability of a disease-specific registry, and finally local government involvement to establish stroke care as a priority.

AUTHOR CONTRIBUTIONS

RV-H and MG are the main authors. They have reviewed the bibliography and draft the manuscript. SA and MS-P have contributed revising the manuscript. AR and GG have contributed managing and analyzing the data presented of the Catalan Stroke Programme.

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Paradigm Shift to Neuroimmunomodulation for Translational Neuroprotection in Stroke

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The treatment of acute ischemic stroke is still an unresolved clinical problem since the only approved therapeutic intervention relies on early blood flow restoration through pharmacological thrombolysis, mechanical thrombus removal, or a combination of both strategies. Due to their numerous complications and to the narrow time-window for the intervention, only a minority of stroke patients can actually benefit from revascularization procedures, highlighting the urgent need of identifying novel strategies to prevent the progression of an irreversible damage in the ischemic penumbra. During the past three decades, the attempts to target the pathways implicated in the ischemic cascade (e.g., excitotoxicity, calcium channels overactivation, reactive oxygen species (ROS) production) have failed in the clinical setting. Based on a better understanding of the pathobiological mechanisms and on a critical reappraisal of most failed trials, numerous findings from animal studies have demonstrated that targeting the immune system may represent a promising approach to achieve neuroprotection in stroke. In particular, given the dualistic role of distinct components of both the innate and adaptive arms of the immune system, a strategic intervention should be aimed at establishing the right equilibrium between inflammatory and reparative mechanisms, taking into consideration their spatio-temporal recruitment after the ischemic insult. Thus, the application of immunomodulatory drugs and their ability to ameliorate outcomes deserve validation in patients with acute ischemic stroke.

Keywords: brain ischemia, immune system, inflammation, ischemic cascade, neuroprotection, stroke

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INTRODUCTION

Over the past three decades, experimental studies, as well as evidence from the clinical setting, have provided the basis for understanding the pathobiological mechanisms underlying stroke. Despite significant advances were made in the elucidation of cellular and molecular pathways implicated in brain ischemia, none of the previously identified potential molecular targets has actually been translated into an effective pharmacological therapy. Therefore, the concept of neuroprotection in stroke is increasingly considered as a chimera, leading to a strong disappointment in all the players involved in the research and development pipeline, from the financial supporters to the basic and

clinical researchers. In fact, the only therapeutic intervention approved for the acute treatment of ischemic stroke patients is based on early blood flow restoration through pharmacological thrombolysis (within 4.5 h from symptoms onset), mechanical thrombus removal (within 6 h from onset) or a combination of both strategies (Saver et al., 2015; Goyal et al., 2016; Rodrigues et al., 2016; Campbell et al., 2017; Shireman et al., 2017). Two very recent clinical trials (DEFUSE3 and DAWN) have revealed that endovascular thrombectomy, initiated up to 16–24 h after patients were last known well, results in significant amelioration of outcomes, especially in patients with small infarct core volumes (i.e., with slow early DWI growth rate) (Albers et al., 2018; Nogueira et al., 2018). Thus, the paradigm "time is brain" deserves a revision, based on a better understanding of the evolution of ischemic core lesions and on imaging-based selection criteria of patients that have been suggested to underlie the "late window paradox" (Sandercock and Ricci, 2017; Albers, 2018).

Although the narrow time-window for the intervention might no longer be considered a major drawback of revascularization approaches (at least in some patients), these procedures are still endowed with a number of disadvantages, such as a relative high percentage of failures and a number of complications that strongly limit their therapeutic use (Gill et al., 2014; Saver et al., 2015; Balami et al., 2017; Kim, 2017). As a result, only a minority of stroke patients are eligible for revascularization procedures and can actually benefit from them. A major issue that needs to be considered relates to the fact that these approaches do not act on the brain tissue to provide neuroprotection, as they do not interfere with the cascade of events that lead to cerebral damage, neither they affect any process that prompts neuronal survival. This highlights the need for a better understanding of the cellular and molecular mechanisms involved in the development of a structural lesion in the ischemic penumbra, where the progression of an irreversible lesion occurs more slowly as compared to the rapidly demising core (Dirnagl et al., 1999; Heiss, 2012; Davis and Donnan, 2014). Reducing the growth rate of the ischemic core represents a pivotal pharmacological goal both to limit the progression of ischemic brain damage and to increase patient eligibility and success rate of endovascular procedures (Lo and Ning, 2016; Albers, 2018).

FROM NEUROPROTECTION TO IMMUNOMODULATION

Cerebral ischemia is typically triggered by the interruption of blood supply to the brain, leading to energy failure, and activation of a cascade of events that ultimately causes brain damage. The ischemic cascade has been thoroughly described elsewhere (Dirnagl et al., 1999; Candelario-Jalil, 2009; Moskowitz et al., 2010; Heiss, 2012). Briefly, as a result of decreased oxygen supply, neurons are unable to accomplish aerobic respiration in mitochondria, intracellular pH decreases, deterioration of membrane ion gradients occurs, and cellular swelling results in cytotoxic oedema (Kohno et al., 1995; Hu and Song, 2017; von Kummer and Dzialowski, 2017). Excitatory neurotransmitters are released in the extracellular space, reaching concentrations that are toxic to neurons. Excitotoxicity, Ca^{2+}-dependent activation of detrimental enzymes, excessive production of reactive oxygen species (ROS) and inflammation represent crucial mechanisms underlying blood-brain barrier (BBB) disruption and neuronal death in the ischemic brain (Benveniste et al., 1984; Rothman and Olney, 1986; Butcher et al., 1990; Bano and Nicotera, 2007; Anrather and Iadecola, 2016; Curcio et al., 2016; Amantea and Bagetta, 2017). At least in experimental model systems, the ischemic insult results in upregulation of diverse programmed cell death pathways, whereby the active crosstalk between apoptosis, necroptosis, and autophagy pathways ultimately affects cellular fate (Yuan and Yankner, 2000; Wang et al., 2018).

During the past three decades, in order to achieve neuroprotection, a large number of studies was aimed at validating these mechanisms as potential therapeutic targets. Despite effective neuroprotection was obtained in the preclinical setting by a number of approaches (i.e., glutamate receptor antagonism, calcium channel blockade, magnesium infusion, free radical scavenging, attenuation of inflammatory responses), there was an overwhelming failure to validate in patients these apparently promising findings. Several reasons have been postulated to explain why neuroprotection has not worked in human stroke, including limitations of the animal models used, erroneous preclinical or clinical trial design and/or inadequate selection of patients (Klehmet et al., 1999; Ford, 2009; Howells et al., 2014). Moreover, most of the studies on neuroprotection in stroke focused on acute mechanisms, occurring quite early after stroke injury, and were primarily aimed at targeting neuron-specific responses (Ginsberg, 2008). During the past decade, a more integrated view of the brain has highlighted the pivotal role of several components of the neurovascular unit in neuronal function and dysfunction (Iadecola, 2017). In fact, a dynamic crosstalk between neurons, glia, endothelium, and blood-borne cells dramatically affects the progression of ischemic brain damage (Lo and Ning, 2016). Due to the discovery that inflammatory mediators play a crucial role in the progression of the damage in the penumbra, more recently, the efforts of stroke researchers were devoted to the identification of neuroprotective candidates through the attenuation of the neuroinflammatory response (Veltkamp and Gill, 2016). In this context, hypothermia has been reported to decrease activation/production of inflammatory mediators in the ischemic brain (Deng et al., 2003; Lee et al., 2016; Sandu et al., 2016). However, the pure blockade of a single inflammatory mechanism has led to disappointing results, being most mediators endowed with dualistic effects on the progression of ischemic brain damage (Amantea et al., 2014). This is consistent with the ability of the brain to trigger regenerative responses that are essential for spontaneous recovery and involve cell genesis, axon growth, and synaptic modulation (Chu et al., 2012; Hermann and Chopp, 2014; Felling and Song, 2015). In this context, astrocytes, microglia, and monocytes/macrophages are among the most potent modulators of brain repair/regeneration (Amantea et al., 2015; Liu and Chopp, 2016). Indeed, a dualistic nature has been ascribed to the immune system, since both the innate

and adaptive responses triggered following an ischemic stroke consist of detrimental or beneficial/reparative components that differentially evolve depending on the spatiotemporal progression of tissue injury (Fumagalli et al., 2015; Gill and Veltkamp, 2016).

THE INVOLVEMENT OF THE IMMUNE SYSTEM

Release of damage-associated molecular pattern molecules upon the ischemic insult, triggers a rapid activation and proliferation of local microglia (Li et al., 2013; Benakis et al., 2014) that acquires an amoeboid phenotype typically associated with phagocytosis that underlies the important task of clearing debris and repairing the tissue (Schilling et al., 2005; Fang et al., 2014; Li et al., 2015). However, microglia activation also results in the release of inflammatory mediators, such as tumor necrosis factor (TNF), and ROS that increase susceptibility to cortical spreading depression (CSD) (Shibata and Suzuki, 2017) and prompt BBB damage, thus fostering the brain recruitment of leukocytes, including monocytes, neutrophils, and T cells (Gelderblom et al., 2009; Chu et al., 2014; Ritzel et al., 2015). It has been suggested that stroke mobilizes immature Ly6C^{hi} inflammatory monocytes that infiltrate the ischemic brain early after injury, reaching the core of the lesion. Then, monocytes progressively acquire the expression of typical markers of alternatively activated M2 macrophages, like arginase-1 and Ym-1, suggesting their possible role in tissue repair during the sub-acute phase of stroke (Miró-Mur et al., 2015). At least in animal models, during the early phases after ischemia, microglia, and macrophages adopt an M2 reparative phenotype, then superseded by ischemia-induced M1-like phenotypes that populate the injured brain days after the initial insult (**Figure 1**; Perego et al., 2011; Hu et al., 2012; Fumagalli et al., 2015; Ritzel et al., 2015; Wattananit et al., 2016; Kronenberg et al., 2017; Greco et al., 2018). These inflammatory phenotypes participate to cerebral injury, by releasing neurotoxic substances [i.e., TNF-α, interleukin (IL)-1β, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, and IL-6] and ROS. To counteract this inflammatory, detrimental process, sublethally injured neurons in the ischemic penumbra produce IL-4, a potent M2-polarizing cytokine (Zhao et al., 2015). In a recent study, selective depletion of different monocyte/macrophage subsets did not influence functional and histological outcomes after 30 min of transient MCAo in rodents, thus suggesting that monocytes/macrophages may not affect damage after a mild ischemic insult (Schmidt et al., 2017). However, that study did not take into account that microglia and monocytes/macrophages experience mixed and complex polarization dynamics dramatically affected by the spatio-temporal production of ischemia-induced microenvironmental stimuli (Fumagalli et al., 2015). Understanding these mechanisms and identifying cellular targets that allow the fine tuning of M1-to-M2 polarization shift represent a promising strategy to implement clinical success of stroke therapy. This approach has been successfully validated in preclinical settings, whereby a number of drugs that reduce the M1/M2 ratio during the acute phase of stroke ameliorate histological and functional outcomes in rodents. Among these, there is evidence that certain antibacterial drugs endowed with strong immunomodulatory properties, such as minocycline and azithromycin, may represent promising candidates as stroke therapeutics (Liao et al., 2013; Yang et al., 2015; Amantea et al., 2016a,b). Similarly, other drugs belonging to different chemical and pharmacological classes share the ability to provide neuroprotection by reducing the M1/M2 ratio, including eplerenone spironolactone (Frieler et al., 2011, 2012), Exendin-4 (Darsalia et al., 2014), metformin (Jin et al., 2014), and rosiglitazone (Han et al., 2015). Intriguingly, the latter drug, as well as bexarotene, have been shown to provide neuroprotection in animal models of focal cerebral ischemia by promoting the polarization of neutrophils toward the beneficial N2 phenotype (Cuartero et al., 2013; Certo et al., 2015).

In fact, similar to other myeloid cells, neutrophils may display different features in stroke, ranging from the inflammatory functions of the N1 phenotype to supportive and beneficial roles for the N2 phenotype (Jickling et al., 2015; Ruhnau et al., 2017). After the ischemic insult, they rapidly release ROS and cytokines that, together with an increased activity of proteases, contribute to BBB rupture, brain oedema, and cerebral damage (Jickling et al., 2015; Frieler et al., 2017). Brain recruitment of neutrophils correlates with poor neurological outcome and brain damage severity both in humans and in rodents. Although there is some evidence arguing that they only accumulate in the perivascular space, without actually penetrating the brain parenchima (Enzmann et al., 2013; Perez-de-Puig et al., 2015), the crucial role of neutrophils in the progression of ischemic cerebral damage is clearly demonstrated (Matsuo et al., 1994; Garcia-Bonilla et al., 2014; Gelderblom et al., 2014; Maestrini et al., 2015; Neumann et al., 2015; Frieler et al., 2017). However, although neutrophils were regarded as promising pharmacological targets for the treatment of ischemic stroke, the success of clinical studies was limited by their beneficial properties (Jickling et al., 2015). Therefore, targeting myeloid cells by blocking their N1- or M1-mediated detrimental functions, while promoting their shift toward N2- or M2-like phenotypes at early times after injury should be considered a reliable strategy for neuroprotection in stroke.

EVOLUTION OF INNATE AND ADAPTIVE IMMUNE RESPONSES

A major advantage of targeting the immune system in ischemic stroke is ascribable to the possibility to widen the time-window for the intervention since immune responses are targetable in the acute, subacute, and chronic phases of recovery. The initial recruitment of local (i.e., microglia) and peripheral innate immune cells (i.e., monocytes/macrophages and neutrophils) underlies the early, non-specific, inflammatory response to the ischemic damage. Upon injury, the brain rapidly communicates with the periphery, thus an exponential increase in neutrophils count and an exponential decrease in the lymphocyte count occur

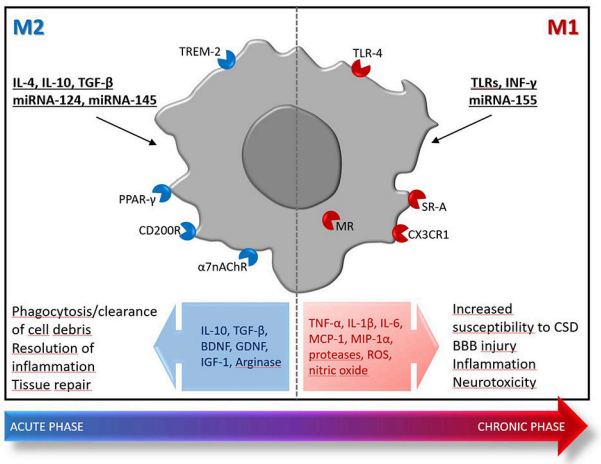

FIGURE 1 | Polarization of microglia/macrophages toward protective (M2) or inflammatory (M1) phenotypes after ischemic stroke. Early after the ischemic insult, locally activated microglia/macrophages and blood-borne monocytes/macrophages display an M2 phenotype characterized by enhanced phagocytic activity and production of mediators [e.g., IL-10, transforming growth factor (TGF)-β, arginase] that provide resolution of inflammation and pro-survival effects toward hypoxic/ischemic neurons. This M2-mediated response is transient and quenches few days after the insult, being replaced by an inflammatory detrimental phase dominated by M1-polarized cells that exhibit reduced phagocytosis ability and release of inflammatory and neurotoxic mediators (e.g., TNF-α, IL-1β, IL-6, MCP-1, MIP-1α, proteases, ROS, nitric oxide). At later stages, release of regulatory and growth factors [e.g., brain-derived neurotrophic factor (BDNF), glia-derived neurotrophic factor (GDNF), and insulin-like growth factor (IGF)-1] by M2-like microglia/macrophages contributes to reparative mechanisms implicated in late tissue recovery. The M1/M2 dichotomy is an illustrative theoretical framework that only embodies two extreme activation states of a spectrum of different functional phenotypes that actually occur in the damaged tissue. The inflammatory M1 phenotypes are typically triggered by interferon (INF)-γ, TLRs modulation or miRNA-155; whereas, the reparative M2 phenotypes are prompted by IL-4, IL-10, TGF-β, miRNA-124, or miRNA-145. Moreover, a number of receptors have been demonstrated to trigger polarization toward the M2 or M1 phenotype, thus representing promising targets for successful immunomodulation in stroke: the α7 nicotinic acetylcholine receptor (α7 nAChR), the CD200R, the peroxisome proliferator-activated receptor-γ (PPARγ), the triggering receptor expressed on myeloid cells (TREM), the class A scavenger receptor (SR-A), the fractalkine receptor CX3CR1 and the mineralcorticoid receptor (MR).

in the hours immediately after stroke onset in patients (Veltkamp and Gill, 2016). Moreover, the abundance of inflammatory monocytes in the blood of stroke patients has been linked to unfavorable outcome (Urra et al., 2009; Kaito et al., 2013).

Genomic profiling of peripheral blood has allowed to identify critical genes and biological immune processes associated with ischemic brain injury in patients (Oh et al., 2012; Barr et al., 2015; Asano et al., 2016). The pathway of innate immunity toll-like receptors (TLRs) has been suggested to be upregulated in the blood of acute ischemic stroke patients within 24 h of symptoms onset (Barr et al., 2010), which is consistent with the evidence that poor outcome is associated with increased expression of TLR-4 in monocytes (Yang et al., 2008; Urra et al., 2009). Accordingly, TLR-4 contributes to brain damage and inflammation in mice subjected to focal cerebral ischemia and promotes haemorrhagic transformation induced by delayed tPA administration (Caso et al., 2007; García-Culebras et al., 2017). At later time-points, i.e., 24–48 h after stroke, the primary pathway expressed in the peripheral blood of stroke patients relies on cytotoxic T-lymphocyte antigen 4 (CTLA4) (Barr et al., 2015), a costimulatory molecule expressed by activated T cells that serves as a negative modulator of adaptive immune cell functions (Buchbinder and Hodi, 2015). Therefore, T cells responsiveness decreases during the first 24–48 h after human stroke, which is consistent with the shift into a suppression state of the adaptive immune response (Miró-Mur et al., 2016). Interestingly, innate immune responses have been shown to have a role in self-tolerance, since the soluble form of CD163, a scavenger receptor shed from the plasma membrane of activated monocytes after stroke, increases in the blood circulation upon injury and exerts inhibitory effects on lymphocytic activity and proliferation (Moeller et al., 2012; Buechler et al., 2013; Lee et al., 2014; O'Connell et al., 2017). Arginase 1, released from circulating neutrophils upon acute ischemic stroke, has also been suggested to contribute to lymphocyte suppression in patients (Asano et al., 2016; Petrone et al., 2016). Notably, stroke-induced immunodepression is characterized by a transient lymphopenia, lymphoid organ atrophy, and monocyte deactivation, a condition that serves to reduce the probability of an autoimmune response toward brain antigens exposed and presented to lymphoid cells after BBB disruption (Urra et al., 2014). However, any potential brain protective effect of stroke-induced immunodepression by attenuating or preventing lymphocyte-mediated brain damage is confounded by stroke severity and by an increased incidence of infections, that represents a major cause of death in the post-acute phase (Miró-Mur et al., 2016). Thus, the maintenance of an adequate equilibrium between self-tolerance and pathogen susceptibility is crucial for patient recovery, whereby the degree of immune suppression requires an adequate balance between innate and adaptive responses. In fact, the disruption of the crosstalk between innate and adaptive mechanisms is harmful and is predictive of poor recovery. As a consequence of this maladaptation, an elevated neutrophil/lymphocyte ratio is used to predict poor prognosis in acute ischemic stroke patients (Brooks et al., 2014; Celikbilek et al., 2014; Xue et al., 2017) and has recently been suggested to be an independent risk factor for ischemic stroke incidence in generally healthy adults (Suh et al., 2017).

The transient suppression of the immune response is followed by sensitization of the peripheral immune system to brain-derived antigens. These latter become unmasked by the insult, as rupture of the BBB and other mechanisms expose reactive peptides that are normally sequestered (Becker, 2009; Urra et al., 2014). By contrast, antigen-independent mechanisms are likely to underlie the deleterious effects of T cells in the very early phase of ischaemia (Gill and Veltkamp, 2016). Thus, an adaptive immune system response builds up through the recruitment of T lymphocytes and natural killer T cells within few days after stroke, followed by a delayed (i.e., persisting for up to 30 days after the insult) activation and proliferation of regulatory T cells (Treg) that restrain the inflammatory response triggered

by acute brain damage (Gill and Veltkamp, 2016). Therefore, likewise the early innate immunity, late T-cell mediated responses are endowed with both inflammatory and protective functions. This may explain the clinical failure to reduce infarct growth of natalizumab, a humanized monoclonal antibody against the glycoprotein α4 integrin expressed on the surface of lymphocytes and monocytes (Elkins et al., 2017). By reducing the adhesion of leukocytes to the endothelial vessel wall, natalizumab blocks their brain infiltration in animal models, resulting in reduced infarct volume after the permanent distal occlusion of the middle cerebral artery, which causes a small cortical infarction (Llovera et al., 2015). The fact that, under certain experimental conditions, natalizumab failed to exert neuroprotection, despite its ability to reduce recruitment of T cells and neutrophils, can be explained on the basis of its specific mechanism of action that does not allow to discriminate different subpopulations of leukocytes, namely the drug also blocks cerebral invasion of potentially protective phenotypes (Langhauser et al., 2014; Tatlisumak, 2017). This further highlights that any intervention aimed at targeting the immune system should selectively suppress detrimental responses, while promoting those that contribute to resolution of inflammation, repair, and regeneration.

CONCLUSIONS

Despite the numerous experimental efforts taken to identify potential pharmacological targets for neuroprotection in stroke, only little progress has been made in translating these findings to the clinical setting. As a consequence, patients can only rely on procedures that allow reperfusion of the occluded vessels, endowed with a number of limitations that maintain ischemic stroke, a leading cause of death and long-term disability worldwide. The results of two recent clinical trials (DEFUSE3 and DAWN) have deranged the "time is brain" paradigm, enhancing the expectations for eligibility of patients for endovascular thrombectomy, especially for those with a slowly expanding ischemic core. This will give new impulse to basic research, since blocking the mechanisms of infarct growth in the penumbra would result in better access to endovascular therapies, as well as in reduced brain damage and neurological deficit. Given the strict interplay between the ischemic penumbra and the innate and adaptive arms of the immune system, growing evidence highlights the promising neuroprotective effects of a rational immunomodulation in stroke models. Thus, re-establishing the equilibrium between inflammatory, detrimental immune responses, and reparative mechanisms represents a promising strategy that deserves validation in patients with acute ischemic stroke.

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All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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Technological Approaches for Neurorehabilitation: From Robotic Devices to Brain Stimulation and Beyond

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Neurological diseases causing motor/cognitive impairments are among the most common causes of adult-onset disability. More than one billion of people are affected worldwide, and this number is expected to increase in upcoming years, because of the rapidly aging population. The frequent lack of complete recovery makes it desirable to develop novel neurorehabilitative treatments, suited to the patients, and better targeting the specific disability. To date, rehabilitation therapy can be aided by the technological support of robotic-based therapy, non-invasive brain stimulation, and neural interfaces. In this perspective, we will review the above methods by referring to the most recent advances in each field. Then, we propose and discuss current and future approaches based on the combination of the above. As pointed out in the recent literature, by combining traditional rehabilitation techniques with neuromodulation, biofeedback recordings and/or novel robotic and wearable assistive devices, several studies have proven it is possible to sensibly improve the amount of recovery with respect to traditional treatments. We will then discuss the possible applied research directions to maximize the outcome of a neurorehabilitation therapy, which should include the personalization of the therapy based on patient and clinician needs and preferences.

Keywords: brain–computer interface, motor impairment, neurologic disorder, neuromodulation, personalization

INTRODUCTION

According to the World Health Organization (WHO), neurological disorders and injuries account for the 6.3% of the global burden of disease (GBD) (1, 2). With more than 6% of DALY (disability-adjusted life years) in the world, neurological disorders represent one of the most widespread clinical condition. Among neurological disorders, more than half of the burden in DALYs is constituted by cerebral-vascular disease (55%), such as stroke. Stroke, together with spinal cord injury (SCI), accounts for 52% of the adult-onset disability and, over a billion people (i.e., about a 15% of the population worldwide) suffer from some form of disability (3). These numbers are likely to increase in the coming years due to the aging of the population (4), since disorders affecting people aged 60 years and older contribute to 23% of the total GBD (5).

Standard physical rehabilitation favors the functional recovery after stroke, as compared to no treatment (6). However, the functional recovery is not always satisfactory as only 20% of patients fully resume their social life and job activities (7). Hence, the need of more effective and patient-tailored rehabilitative approaches to maximize the functional outcome of neurological injuries as well as patients' quality of life (8). Modern technological methodologies represent one of the most recent advances in neurorehabilitation, and an increasing body of evidence supports their role in the recovery from brain and/or medullary insults. This manuscript provides a perspective on how technologies and methodologies could be combined in order to maximize the outcome of neurorehabilitation.

CURRENT SYSTEMS AND THERAPEUTIC APPROACHES FOR NEUROREHABILITATION

The great progress made in interdisciplinary fields, such as neural engineering (9, 10), has allowed to investigate many neural mechanisms, by detecting and processing the neural signals at high spatio-temporal resolution, and by interfacing the nervous system with external devices, thus restoring neurological functions lost due to disease/injury. The progress continues in parallel to technological advancements. The last two decades there has seen a large proliferation of technological approaches for human rehabilitation, such as robots, wearable systems, brain stimulation, and virtual environments. In the next sections, we will focus on: robotic therapy, non-invasive brain stimulation (NIBS), and neural interfaces.

Robotic Devices

Robots for neurorehabilitation are designed to support the administration of physical exercises to the upper or lower extremities, with the purpose of promoting neuro-motor recovery. This technology has a relatively long history, dating back to the early 1990s (11). Robot devices for rehabilitation differ widely in terms of mechanical design, number of degrees of freedom, and control architectures. As regards the mechanical design, robots may have either a single point of interaction (i.e., end effector) with the user body (endpoint robots or manipulanda) or multiple points of interaction (exoskeletons and wearable robots) (12).

Endpoint robots for the upper extremity, include Inmotion2 (IMT, USA) (13), KINARM End-Point (BKIN, Canada), and Braccio di Ferro (14) (**Figure 1A1**, left). Only some of these devices have been tested in randomized clinical trials (15), confirming an improvement of upper limb motor function after stroke (16). However, convincing evidence in favor of significant changes in activities of daily living (ADL) indicators is lacking (17), possibly because performance in ADL is highly affected by hand functionality. A good example of lower limb endpoint robot is represented by gait trainer GT1 (Reha-Stim, Germany). Its efficacy was tested by Picelli et al. (18), who demonstrated an improvement in multiple clinical measures in subjects with Parkinson's disease following robotic-assisted rehabilitation when compared to physical rehabilitation alone (18). Endpoint robots are also available for postural rehabilitation. For instance, Hunova (Movendo Technology, Italy, launched in 2017) is equipped with a seat and a platform that induce multidirectional movements to improve postural stability (**Figure 1A1**, right).

Typical lower limb exoskeletons range from large systems, equipped with treadmill and weight support, and intended for hospital use, like the Lokomat (Hocoma, Switzerland) and the LOPES system (26), to more lightweight devices intended for overground walking, like Ekso (Ekso Bionics, USA), Indego (Parker Hannafin, USA), Rewalk (Rewalk Robotics, USA), and the most recent one, Twin (IIT-INAIL, Italy). Notably, Twin has been developed according to long interactions with focus groups of disabled patients (**Figure 1A2**). A few exoskeletons for the upper limb have also been developed. They also range from lab systems—e.g., the KINARM Exoskeleton (BKIN, Canada) or the Armeo Spring and Power (Hocoma, Switzerland)—to wearable, modular devices (27–29).

One common feature of rehabilitation robots, is that they are equipped with movement and/or force sensors, so that they integrate functionalities both for the assessment [i.e., quantify users' movements and exchanged forces (30)] and the treatment (i.e., administer highly reproducible, repetitive exercise protocols, and interaction modalities).

In spite of the increasing volume of published studies, the number of high-quality clinical trials on robot-assisted therapy is still relatively low. A large multi-center RCT comparing robot therapy, intensive physical therapy, and usual care (31) confirmed that robots are indeed effective, but found no significant advantage over conventional physical therapy. A systematic comparison of different approaches (32) suggested that robot therapy is among the most effective techniques for the rehabilitation of both upper and lower limbs. Moreover, recent studies concluded that robot-assisted gait training in combination with physiotherapy is more likely to achieve independent walking than gait training alone (33, 34).

A major limitation of endpoint robotic approach is that the improvement is limited to the body regions involved in training. In a clinical setting, robotic rehabilitation may be cost and time-consuming, and for this reason, it is difficult to imagine the combination of different endpoint robotic devices in the patient who have an impairment that affects multiple body areas, i.e., post-stroke hemiplegia. Moreover, early robots for neuroehabilitation were specifically aimed at substituting labor-intensive physical rehabilitation with minimal human intervention, producing an automatic and repetitive treatment. This initial trend, however, minimizes the importance of both therapist knowledge and patient–physician relationship. However, the ability to precisely quantify sensorimotor performance during exercise in terms of movement kinematics and exchanged forces is leading to a new revolution in rehabilitation, toward evidence-based and knowledge-driven approaches. Modern rehabilitation devices automatically adapt task difficulty and assistance modalities to individual performance (35). In the future, they may incorporate models of the recovery process (36) to predict the rehabilitation outcome (37) that will be fitted on patient's features.

Another stimulating challenge is the development of lightweight robots suitable for the use outside of the hospitals, in domestic or community environments and in conjunction with

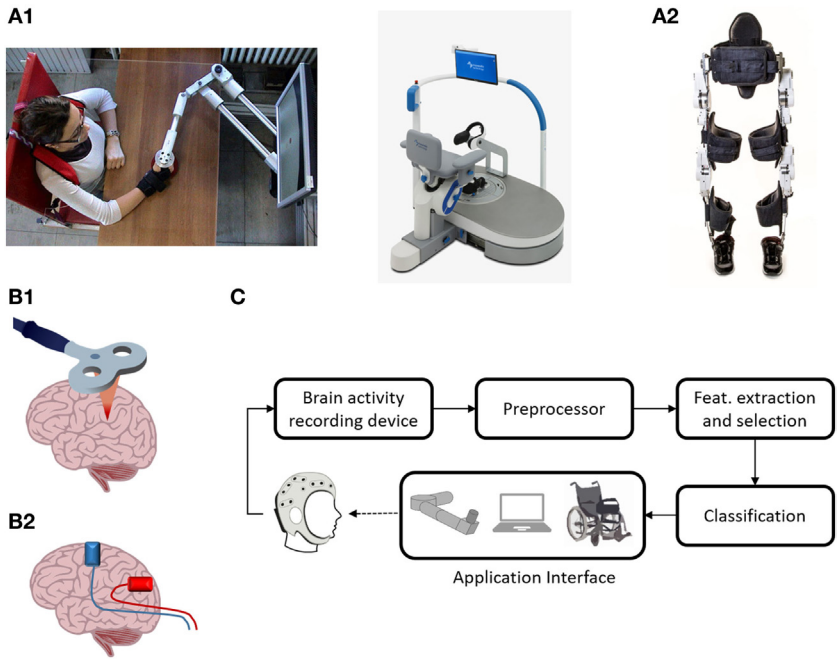

FIGURE 1 | Neurorehabilitation therapies. **(A1)** Endpoint robots: on the left the "Braccio di Ferro" manipulandum, on the right the postural robot Hunova. Braccio di ferro (14) is a planar manipulandum with 2-DOF, developed at the University of Genoa (Italy). It is equipped with direct-drive brushless motors and is specially designed to minimize endpoint inertia. It uses the H3DAPI programming environment, which allows to share exercise protocol with other devices. Written informed consent was obtained from the subject depicted in the panel. Movendo Technology's Hunova is a robotic device that permits full-body rehabilitation. It has two 2-DOF actuated and sensorized platforms located under the seat and on the floor level that allow it to rehabilitate several body districts, including lower limb (thanks to the floor-level platform), the core, and the back, using the platform located underneath the seat. Different patient categories (orthopedic, neurological, and geriatric) can be treated, and interact with the machine through a GUI based on serious games. **(A2)** Wearable device: the recent exoskeleton Twin. Twin is a fully modular device developed at IIT and co-funded by INAIL (the Italian National Institute for Insurance against Accidents at Work). The device can be easily assembled/disassembled by the patient/therapist. It provides total assistance to patients in the 5–95th percentile range with a weight up to 110 kg. Its modularity is implemented by eight quick release connectors, each located at both mechanical ends of each motor, that allow mechanical and electrical connection with the rest of the structure. It can implement three different walking patterns that can be fully customized according to the patient's needs *via* a GUI on mobile device, thus enabling personalization of the therapy. Steps can be triggered *via* an IMU-based machine state controller. **(B1)** Repetitive transcranial magnetic stimulation (rTMS) representation. rTMS refers to the application of magnetic pulses in a repetitive mode. Conventional rTMS applied at low frequency (0.2–1 Hz) results in plastic inhibition of cortical excitability, whereas when it is applied at high frequency (≥5Hz), it leads to excitation (19). rTMS can also be applied in a "patterned mode." Theta burst stimulation involves applying bursts of high frequency magnetic stimulation (three pulses at 50 Hz) repeated at intervals of 200 ms (20). Intermittent TBS increases cortical excitability for a period of 20–30 min, whereas continuous TBS leads to a suppression of cortical activity for approximately the same amount of time (20). **(B2)** Transcranial current stimulation (tCS) representation. tCS uses ultra-low intensity current, to manipulate the membrane potential of neurons and modulate spontaneous firing rates, but is insufficient on its own to discharge resting neurons or axons (21). tCS is an umbrella term for a number of brain modulating paradigms, such as transcranial direct current stimulation (22), transcranial alternating current stimulation (23), and transcranial random noise stimulation (24). **(C)** A typical BCI system. Five stages are represented: brain-signal acquisition, preprocessing, feature extraction/selection, classification, and application interface. In the first stage, brain-signal acquisition, suitable signals are acquired using an appropriate modality. Since the acquired signals are normally weak and contain noise (physiological and instrumental) and artifacts, preprocessing is needed, which is the second stage. In the third stage, some useful data or so-called "features" are extracted. These features, in the fourth stage, are classified using a suitable classifier. Finally, in the fifth stage, the classified signals are transmitted to a computer or other external devices for generating the desired control commands to the devices. In neurofeedback applications, the application interface is a real-time display of brain activity, which enables self-regulation of brain functions (25).

ADL, e.g., over ground walking in unstructured environments. This implies a modular structure, which facilitates donning and transportability.

Non-Invasive Brain Stimulation

Non-invasive brain stimulation techniques are a promising adjuvant strategy for enhancing post-injury recovery. In recent years, more than 1,400 studies were performed in humans, with at least one-fifth of these focusing on stroke rehabilitation. NIBS techniques involve modulation of the central nervous system by electrically activating neurons in the brain (38) and can be used to influence cortical excitability, neuroplasticity, and behavior (39, 40). Repetitive transcranial magnetic stimulation (rTMS, **Figure 1B1**) and transcranial current stimulation (tCS, **Figure 1B2**) are the most common and widely used techniques (39). Because of its relative ease of use, portability and decreased safety risk compared to rTMS, tCS is emerging as an effective and versatile clinical tool to prime the brain activity prior to or during neurorehabilitation. Starting from the hypothesis on training-induced plasticity, NIBS could be applied to foster plasticity induction, also in the spinal cord as shown in animals (41, 42) and in humans (43).

Related to rehabilitation, one of the major challenges is to design interventions that are efficient, promote motor learning, consolidate skills, and augment retention. For example, NIBS approach to stroke rehabilitation has focused on excitation of the unaffected hemisphere, of the affected hemisphere, or inhibition of unaffected hemisphere, also combining neuromodulation of both hemispheres (44). To date, a number of sham-controlled studies based on NIBS have been performed, but the evidence remains inconsistent. A Cochrane review failed to support the efficacy of rTMS for stroke rehabilitation (45), although other studies (46, 47) concluded that low frequency rTMS was effective in improving ADL and aphasia. A recent review (48) concluded that rTMS may produce both short- and long-term improvement on motor recovery in stroke patients, in particular when neuromodulation is initiated early after stroke, and with better results in case of sub-cortical lesions with respect to cortical ones. As regards tCS, it appeared to be useful for motor recovery in a sub population of patients with chronic stroke and low functional impairment (49) and very well tolerated (50), but a Cochrane review (51) failed to support its effectiveness. A possible explanation for these inconsistent conclusions is the lack of a correct patient stratification, and thus a tailored stimulation protocol (51, 52).

Some ethical and technical considerations deserve discussion. First, use of NIBS, calls for greater caution on pediatric population, given the higher stakes and uncertain future effects for brains still undergoing rapid and formative development (53). Second, a careful evaluation of the use of NIBS must also be warranted in adults, regarding informed consent and patient selection (54). Any direct interference with neural activity, even beneficial, might be described more accurately as "minimally invasive" (55). Moreover, when considering that most of the studies mainly focused on the short-term, short-lasting effects of NIBS, it is important to evaluate the long-term effects of modulating cortical electric fields in patients with cortical impairment.

Careful monitoring is particularly important when considering that, despite researchers' discussion of and explicit warnings against unsupervised use of NIBS (56), brain stimulation products are already commercially available and without proper guidance or information. Thus NIBS could be conducted carelessly with unknown and potentially harmful effects.

Neural Interfaces

In recent years, it is possible to include also neural interfaces among the strategies for neurorehabilitation and indeed the use of these systems in clinical applications is increasing (57, 58).

A neural interface is essentially a system mediating the communication between the brain and an external device (59, 60). Several modalities have been used for brain signal acquisition (61), which include electroencephalography (EEG) (62), magnetoencephalography (MEG) (63), functional magnetic resonance imaging (64), and functional near-infrared spectroscopy (65). Among neural interfaces, the so-called "BCIs" (**Figure 1C**) were essentially conceived as communication tools for paralyzed or locked-in patients (62) and were mainly based on the use of the processed EEG signal. Typical BCI techniques include the use of evoked potentials (such as P300) (66) or motor imagery (67), and enable the user to communicate with a speller device (68) or to control the movement of an end effector, either virtual (69) or real (70). From a clinical point of view, the BCI approach proves to be beneficial in potentiating the impaired motor function, as demonstrated for stroke (71, 72). MEG BCI training allowed patients with chronic stroke to voluntary modulate the μ-rhythm amplitude over the affected hemisphere with the possibility to voluntary control grasping using a robotic hand orthosis (73). More recently, a BCI-orthosis training was tested as add-on to physical therapy in a sham-controlled study (74); after an EEG BCI training protocol the strength in hand muscles significantly improved when compared to sham group. Noteworthy, the results of the above cited studies were achieved in patients in a chronic stage, for whom very limited possibilities are available if treated with standard rehabilitative care. The motor improvement is a consequence of the cortical changes occurring during the interaction with the controlled object, as demonstrated both with invasive and non-invasive studies (75, 76). This promising evidence made BCIs appealing for different types of neurorehabilitation practices, not only in presence of motor disability, but also for the recovery of impaired cognitive functions (76–80). In this framework, a particular form of BCI is that of neurofeedback, in which neural data are visually displayed to the user (81). This technique has proven to be mainly effective in the treatment of attention deficits/hyperactivity, but also for other cognitive dysfunctions (82, 83) and in stroke (84, 85).

Over the past decade also invasive brain-machine interfaces and neural prostheses in general have been the subject of extensive research with promising findings for the treatment of neuro-related impairments (86). The development of these devices will hopefully have a profound social impact on the quality of life, although translation to clinical application is far to be implemented due to the technological barriers (e.g., wired systems or limited bandwidth for wireless systems) and to the limits imposed by the invasiveness of the procedure (e.g., tissue reaction to the brain implant) (87).

Neural prosthesis can be combined with functional electrical stimulation (88, 89). In this scenario, the use of a controlled end effector is substituted by direct stimulation of the involved muscles, therefore, natural movements are recreated by bridging two areas disconnected because of the impairment/disease (88, 89). A system was recently developed allowing a quadriplegic patient chronically implanted with microwire arrays to move the arm by means of muscle stimulation triggered by the recorded and decoded brain signals (90).

Examples of latest-generation neural prostheses involve direct stimulation of central or peripheral neural tissue. Recent animal studies demonstrated locomotion restoration after SCI by spatiotemporal modulation of the spinal cord (91) and restoration of motor function after stroke by activity-dependent stimulation of the motor cortex (92). Whereas, recent human studies demonstrated the restoration of hand reaching and grasping by non-invasive neuromuscular stimulation of hand muscles (93) and prosthetic hand control by invasive stimulation of peripheral nerve (94). In addition, faster and more effective closed loop stimulation protocols are being investigated also in *in vitro* preparations (95).

CHALLENGES AND OPEN ISSUES

We have so far presented the main methodologies for neurorehabilitation and, for each field, the most innovative trends currently under investigation. However, novel rehabilitation approaches are characterized by a synergistic tactic, in which these techniques are used in combination and also mixed with kinematic information (from the robot) and patients' biosignals, such as EEG or EMG (electromyography) (**Figure 2A**).

An example of such a multimodal approach was shown to the general public during the world cup in 2014, when the kick off was given by a paraplegic man using a lower limbs exoskeleton controlled by brain activity. Two years later, it was demonstrated that the combined use of gait rehabilitation with a BMI was able to induce partial neurological recovery in paraplegic patients (96). This represents a valid proof-of-concept for the combination of robotic devices driven by neural activity. Moreover, the number of clinical-oriented versions of this approach is increasing: exoskeletons powered by BCI have been used during post stroke rehabilitation (97, 98). Similar results were obtained with a BCI system for locomotion rehabilitation, based on the use of an avatar in a virtual reality environment (99).

Experiences where assisted locomotion has been used in conjunction with neuromodulation are already present in the literature. Spinal tDCS was applied in patients with SCI undergoing assisted locomotion using driven gait orthosis (Lokomat, Hocoma AG, Volketswil, Switzerland) (100). Results showed that anodal spinal tDCS and assisted locomotion increased spinal reflexes amplitude, suggesting functional effects when the spinal cord is detached from the rest of the central nervous system. These findings open an important avenue of research designed to rescue residual spinal functions by spinal tDCS in SCI patients (100).

Although the combined effect of neuromodulation with robotic therapy still needs to be clinically validated (101), it clearly shows the combined direction of neuromodulation-based rehabilitation. Explorative directions in neuromodulation also include a combination with traditional BMI approaches (102) as well as investigation of non-classical stimulation sites (103). Another potentially useful synergy in the rehabilitative field is represented by the association of biofeedback (104, 105) with robotic rehabilitation (106, 107).

Besides the technological and scientific improvement of neurorehabilitative treatment, a very important trend followed by current research is that of a *personalized treatment*. This is not just intended to focus on a particular disease and address the symptoms shared by different populations of patients, but is truly envisioned as a personalized method for a single individual. The idea of a patient-tailored approach is not new: standardized algorithms have been proposed for stroke, based on the clinical history of the patient, time elapsed after insult, topography of the lesion, type, and severity of functional impairment (108). In this view, it will be desirable to identify solid biomarkers not only in the acute settings, but also in the middle and chronic stages of neurological diseases. This modern approach, recently named as "Rehabilomics," will be useful not only for outcome prediction, but also to foresee the best personalized rehabilitative treatment. Well known biomarkers in stroke are represented by measures of function and structure through neuroimaging after stroke (109) and by biochemical dosages (for example, uric acid, Cu/Zn superoxide dismutase, and urinary 8-OHdG) (110, 111). The technological improvement will help to identify novel biomarkers in neurorehabilitation. For example, a non-linear, composite, model made of robotic measurement in the upper limb was able to predict motor recovery at 90 days from stroke (112). "Technological" measures seem to be complementary rather than substitutive to standard biomarkers (113).

Overall, there is a great need for the development and testing of novel innovative interventional strategies individually tailored to patients' prerequisites. The neurorehabilitation scientific community is finally showing an effort in this direction, by taking into account patients' specific requests (**Figure 2B**). For example, during the sixth International Brain–Computer Interface Meeting in 2016 (114), a virtual forum of BCI users was presented, allowing patients to remotely participate at the conference sessions and also to send video-message with their views and requirements in order to help the scientists shaping the future research directions. This is exactly the approach that should be taken when designing

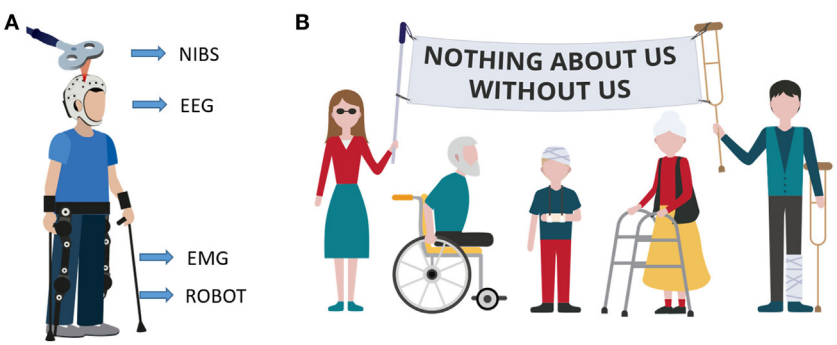

FIGURE 2 | Innovative patient-tailored approach. **(A)** Example of multimodal rehabilitative approach. Subject is using an exoskeleton while receiving brain stimulation. Both exoskeleton motors and stimulation parameters are updated based on subject's biofeedback signals (electroencephalography and/or EMG) and on subject performance (ROBOT) while, at the same time brain stimulation non-invasive brain stimulation and exoskeleton assistance (ROBOT) influence the biosignals. **(B)** Motto of the disabled population. The motto means that any choice (in any field) regarding them must be taken with their direct participation. Rehabilitation research must follow the same policy.

a therapy or experimental protocol targeting a specific set of population. The patients' motto "Nothing about us, without us" clearly indicates that patients must be involved in experimental studies, since the very beginning.

CONCLUSION

In the coming years, science and medicine have to create a integrated dialog with patients, since they will be the first end-users of any technological development. To date, important advances have been made in robotic-based therapy, NIBS and neural interfaces, as integrations and/or alternatives to standard therapy. However, in order to be really effective, neurorehabilitation research must be primarily person-centered (i.e., "personalized"). Personalization calls for flexible solutions, such as combining the main technologies, in order to adapt to the different patient's features and preferences. And this is exactly the direction which should be undertaken in neurorehabilitation.

AUTHOR CONTRIBUTIONS

MS, LDM, and MC conceived the manuscript. MC and LDM coordinated the activities. MS and MC prepared the figures. MS, MC, RDI, and LA revised the manuscript. All the authors wrote and approved the final version of the manuscript.

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Epilepsy an Update on Disease Mechanisms: The Potential Role of MicroRNAs

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So far, research on epilepsy mechanisms has been designed mainly using animal models and tracking down molecular mechanisms underlying seizures in that model. While this approach is clearly valuable, it can be questioned if it is the best possible. One attractive alternative approach may stem from the consideration of epilepsy as a complex disease of a very complex organ, the brain. This short review summarizes data from analyses of the alterations in expression of microRNAs and their target messenger RNAs in a specific brain subregion, the dentate gyrus of the hippocampus, in three experimental models of lesional epilepsy. The findings are discussed within the conceptual framework of complex systems.

Keywords: epilepsy, hippocampus, microRNA, gene expression, meta-analysis

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INTRODUCTION: DEFINITION AND CLASSIFICATION OF THE EPILEPSIES

The term "epilepsy" refers to a collection of diseases of different etiologies, characterized by the spontaneous and unpredictable occurrence of seizures, i.e., of transient "signs and/or symptoms due to abnormal excessive and synchronous neuronal activity in the brain" (1). In practical terms, epilepsy is identified "by any of the following conditions: (1) at least two unprovoked (or reflex) seizures occurring more than 24 h apart; (2) one unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (60% or more) after two unprovoked seizures, occurring over the next 10 years; (3) diagnosis of an epilepsy syndrome" (1).

The classification of the seizures and of the epilepsies has been recently revised by the International League against Epilepsy (2, 3). According to this new classification, seizures are subdivided in focal onset, "when originating within networks limited to one hemisphere"; generalized onset, when "originating at some point within, and rapidly engaging, bilaterally distributed networks"; and unknown onset (2). Focal onset seizures can be further classified based on awareness (focal aware seizures and focal seizures with impaired awareness); based on the motor component (motor and non-motor seizures); based on clinical evolution (focal to bilateral tonic–clonic seizures). Generalized onset seizures can be further classified based on the motor component: motor (tonic–clonic and other types of motor seizures) and non-motor (for example absence seizures).

The epilepsies are classified on the basis of the onset of seizures in focal, generalized, combined focal and generalized, and unknown. Their etiology can be genetic, structural, metabolic, infectious, immune, or again unknown (3).

PATHOGENETIC MECHANISMS

Experimental and clinical studies in genetic and lesional epilepsies suggest the existence of several common pathogenetic mechanisms, which include alterations in the intrinsic properties of the neuron and unbalance between excitatory (glutamate) and inhibitory (γ-aminobutyric acid, GABA) signals (4, 5). Regarding the former, genetic mutations or acquired functional alterations in Na^+ and K^+ channels have been identified in relevant animal models and patients with different forms of epilepsy. Regarding the latter, genetic mutations or acquired functional alterations leading to loss of function of GABAergic neurotransmission (alterations in GABA synthesis, release, or receptors) or gain of function of glutamatergic neurotransmission (alterations in glutamate receptors or reuptake) have been identified.

These findings are coherent with the known mechanisms of action of the currently available anti-epileptic drugs (AEDs (5)). In fact, many AEDs target ion channels and inhibit neuronal excitability. These targets include voltage-gated Na^+ channels (for phenytoin, carbamazepine, valproic acid, felbamate, rufinamide, lamotrigine, lacosamide, topiramate, zonisamide, and oxcarbazepine), K^+ channels (for retigabine) and T-type Ca^{2+} channels (for ethosuximide and valproic acid). Other AEDs target molecules at the excitatory synapse and reduce their efficiency. These targets and drugs include the synaptic vesicle glycoprotein 2A (for levetiracetam), the α2δ subunit of the voltage-gated Ca^{2+} channel (for gabapentin and pregabalin), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid, and N-methyl-D-aspartate receptors (respectively, for perampanel and felbamate). Finally, some AEDs target inhibitory synapses and enhance their efficiency. These include tiagabine, which inhibits the GABA transporter GAT1, leading to reduced GABA uptake into presynaptic terminals and astrocytes and vigabatrin, which irreversibly inhibits the catabolic enzyme GABA transaminase, leading to accumulation of GABA in presynaptic terminals and astrocytes. The benzodiazepines and the barbiturates enhance inhibitory neurotransmission by allosterically modulating $GABA_A$ receptor-mediated Cl^- currents. Incidentally (and interestingly), many AEDs (for example, valproic acid, lamotrigine, phenobarbital, gabapentin, felbamate, and topiramate) can act through multiple complementary mechanisms.

UNMET THERAPEUTIC NEEDS IN EPILEPSY

In spite of all the abovementioned AEDs, many therapeutic needs remain unmet in epilepsy (6, 7). First, current drug treatments must be taken daily even in cases when seizures are rare, and patients may even become paradoxically more exposed to the side effects of their drugs than to the signs and symptoms of their epilepsy. We need more tolerable treatments that do not impact quality of life. Second, the available AEDs can prevent seizures in about two thirds of the patients and are ineffective in some forms of epilepsy. Therefore, we need new treatments for therapy resistance patients. In addition, the available AEDs are actually purely symptomatic agents (8) that, at best, prevent or reduce seizures but do not attenuate some comorbidities (such as depression, cognitive slowing, and memory impairment), which can have a dramatic impact on the quality of life of people with epilepsy (9). Finally, another urgent therapeutic need is the identification of currently unavailable antiepileptogenic and disease modifying therapies. As stated, AEDs are actually symptomatic agents for seizures, but cannot prevent the development of epilepsy in at-risk individuals who experienced a head trauma, an episode of status epilepticus (SE), a stroke, or other epileptogenic insults.

IDENTIFICATION OF DISEASE MECHANISMS

In summary, we do need to move beyond current knowledge and concepts in order to identify disease mechanisms that represent relevant therapeutic targets for improving not only seizures but also epilepsy comorbidities. So far, research on epilepsy mechanisms has been designed mainly using animal models and tracking down molecular mechanisms underlying seizures in that model. While this approach is clearly valuable, it can be questioned if it is the best possible. In fact, it has been postulated that some forms of epilepsy (so-called "system epilepsies") depend on a dysfunction of neuronal systems and not of specific, individual elements of the systems (10). Therefore, one attractive new approach to attempt understanding the mechanisms of epilepsy may stem from the consideration of epilepsy as a complex disease of a very complex organ, the brain (11).

By definition, a complex system is "any system featuring a large number of interacting components, whose aggregate activity is non-linear (i.e. cannot be derived from the summation of the activities of the components) and typically exhibits hierarchical self-organization" (12). Therefore, the brain is a quintessential complex system. In fact, it displays a hierarchical self-organization: molecular networks in each particular cell; neurons and non-neuronal cells organized in local circuits; neuronal networks involving multiple brain areas. At all hierarchical levels, the aggregate activity of the components is non-linear and emerging properties are observed that cannot be inferred from the properties of the single components.

Traditional research approaches tend to assume a linear progression from a specific molecule to cells to integrated function. In contrast, new therapeutic approaches may be developed considering the brain as a complex network and taking into account the non-linearity of its function. The feasibility and the potential of network science applied to regulation of gene expression, activity of local neural network, and global brain function begin to be appreciated (11).

In this line of reasoning, we thought to investigate the modifications of expression of microRNAs (miRNAs) in a specific neuronal population, the dentate gyrus (DG) granule cells (GCs) of the hippocampus, in the natural history of disease in experimental models of post-SE epilepsy (13).

WHY FOCUSING ON miRNAs AND ON DG GCs

MicroRNAs are small size (21–25 nucleotides) endogenous non-coding RNAs. Their main function is to degrade or repress

translation of specific target messenger RNAs (mRNAs), i.e., to regulate their expression at the post-transcriptional level (14, 15). miRNAs and their mRNA targets follow a "many-to-many" mode of interaction, because each miRNA can regulate many mRNAs and each mRNA can be regulated by many miRNAs (16). More than 2,000 human miRNAs have been identified thus far and more than 50% of these are expressed in the brain. miRNAs are implicated in many brain functions that are relevant for epilepsy and epileptogenesis, including cell death, neurogenesis, and synaptic plasticity (17). In fact, several studies support the notion that miRNAs play a pathogenic role in acquired epilepsies (18, 19). Thus, defining the patterns of changes in the miRNA levels in the natural history of epilepsy and their relationship with mRNA changes seems a logical approach to attempt identifying ways to modulate neuronal function in a manner that may be more closely related to the pathogenesis of disease than simply acting on phenomenological aspects such as seizures.

However, alterations in gene expression patterns are cell specific, and performing molecular analyses on large samples of tissue containing multiple cell types generates data that are difficult to interpret, because the cell composition of the same brain area may dramatically change in the course of epilepsy due to cell death, neurogenesis, astrocytosis, and other events (20). Therefore, we decided to start analyzing a specific cell population, the DG GCs. These cells have been considered for long as a "gate" inhibiting hippocampal hyperexcitation (21). Recent evidence reinforced this hypothesis: first, optogenetic GC hyperpolarization stopped spontaneous seizures, whereas GC activation exacerbated seizures; and second, acute seizures were evoked in non-epileptic animals by optogenetic activation of GCs (22). In addition, DG GCs undergo relevant functional changes with epilepsy development, like sprouting of the mossy fibers and increased excitation (20). The DG is very often involved in seizure generation in patients with temporal lobe epilepsy, one of the most common epileptic syndromes in adults, and very often surgically removed in drug resistant patients. Finally, and quite relevant from a practical point of view, DG GCs are a compact layer of almost identical cells, easy to dissect from the rest of the tissue, and very well characterized from a developmental, neurochemical, morphological, and physiological point of view.

miRNA PROFILES IN HIPPOCAMPAL GCs OF RATS WITH PILOCARPINE-INDUCED EPILEPSY AND COMPARISON WITH HUMAN EPILEPTIC SAMPLES

We laser-microdissected the hippocampal granule cell layer (GCL) of rats killed at different time points in the development of pilocarpine SE-induced epilepsy: latency (i.e., the time period between an epileptogenic insult and the first spontaneous seizure), first spontaneous seizure, and chronic epileptic phase. By performing miRNA microarrays on these samples, we identified 63 miRNAs that were differentially expressed at one or another time point. Distinct clusters of miRNAs separated control and chronic phase rats from those sacrificed during latency or after the first spontaneous seizure. Those miRNAs that were altered in the chronic phase of the rat model were then compared with those obtained from the laser-microdissected GCL of temporal lobe epilepsy patients who underwent respective surgery, allowing the identification of many miRNAs (miR-21-5p, miR-23a-5p, miR-146a-5p, and miR-181c-5p) that were upregulated both in the human and in the rat epileptic tissue (13, 23).

META-ANALYSIS OF THE AVAILABLE DATA SETS

Taken together, these results identify miRNA expression patterns that are associated with different phases in the natural history of epilepsy in the pilocarpine model, revealing molecular pathways that may underlie the development and the maintenance of the disease. While these miRNAs represent attractive therapeutic targets, an obvious question arises: are they model- or disease-specific? The overlaps with the human findings in the chronic stage support the notion that animal data can (at least in part) predict the human situation. In addition, an overlap could also be observed between miRNAs differently expressed in this study and those identified in other studies that employed other epilepsy models (24, 25). Thus, these particular miRNAs, whose expression changes are similar in the different data sets, are particularly attractive candidates for further investigation. However, the different miRNA studies vary in many parameters, including the brain region, the animal model, the sample size, the microarray platform, and analysis techniques. Last but not least, each of these studies is underpowered to detect relatively small changes in miRNA expression.

Therefore, to pursue a rigorous answer to the question, we undertook a meta-analysis of the changes in miRNA expression reported to occur in the DG following different epileptogenic insults (26). While meta-analyses of human research data are well established, meta-analyses of preclinical data are rare, primarily because of the small sample sizes and of the high heterogeneity between studies. Indeed, our meta-analysis was the first to be performed on preclinical epilepsy research data.

Inclusion criteria were miRNA array studies in the DG of epileptic vs. control animals. Exclusion criteria were miRNA array studies in large brain areas (including the whole hippocampus). These criteria identified three data sets that used models of epilepsy induced by different epileptogenic insults: focal electrical stimulation of the lateral nucleus of the amygdala (24); focal electrical stimulation of the angular bundle, the main afferent pathway to the hippocampus from the entorhinal cortex (25); and systemic administration of the convulsant agent pilocarpine (13). All these models are characterized by recovery from the initial insult and onset of spontaneous recurrent seizures after a latency period of apparent well being. They all imply a key involvement of the DG but *via* different pathways: direct DG activation with the angular bundle stimulation, indirect with the amygdala stimulation, and widespread activation of the brain in the pilocarpine model (27). All these three studies were underpowered to detect modest fold changes (<2.0) in miRNA expression.

We found that 176 miRNAs were detectable at some time point in any of the three studies. Therefore, we meta-analyzed the expression values of these 176 miRNAs, and identified 26 that were differentially expressed in the latent period and 5 in chronic epilepsy (**Table 1**). Thirteen of these were not identified in the individual studies. In the attempt to begin understanding the role of these 31 miRNAs, their mRNA targets were first predicted *in silico* using the miRWalk database, yielding (as expected) a very large number of potential targets. We then filtered these predicted targets by searching for an inverse relationship with mRNAs identified in separate epileptogenesis studies (28). We found an inverse relationship between 22 (of 26) miRNAs and 112 predicated gene targets. In addition, an inverse relationship was found between 5 (of 5) miRNAs and 29 predicated gene targets for the chronic phase in the only model that could be assessed for this phase (amygdala stimulation).

Analysis was further refined focusing on the epileptogenesis phase. We found that anti-correlated miRNAs and mRNAs may be part of pro- or anti-epileptic mechanisms. For example, the mRNA encoding the mitogen-activated protein kinase kinase kinase 4 was predicted target and had inverse expression relative to four miRNAs (miR-92b-3p, miR-101a-3p, miR-153-3p, and miR-3575-3p), and the mRNA coding for Map3k14 was inversely correlated with two other miRNAs (miR-7a-5p and miR-138-5p). In both these cases, downregulation of miRNAs was associated with upregulation of target genes that are thought to play a pro-epileptic role (29–33). Other anti-correlated miRNAs and mRNAs may instead be part of anti-epileptic mechanisms. For example, synapsin type 2 was inversely correlated with three miRNAs (miR-101a-3p, miR-139-5p, and miR-551b-3p), and the protein tyrosine phosphatase, non-receptor type 5 was inversely correlated with two miRNAs (miR-150-5p and miR-383-5p). In these cases, downregulation of miRNAs and upregulation of the target gene may play an anti-epileptic role (34–38).

Other transcripts that were downregulated during epileptogenesis were predicted targets of miRNAs that were upregulated. For example, the downregulated 5-hydroxytryptamine receptor 5 was a predicted target of the upregulated miR-146a-5p. The downregulated GABA receptor subunit delta was a predicted target of and inversely correlated with miR-212-5p. These changes may play a pro-epileptic role (39, 40).

Finally, we generated a network of miRNAs based on their ability to target common pathways and hypothesized a connection between pairs of miRNAs if the respective mRNA targets belonged to the same, significantly enriched pathways or gene ontology terms. In line with other findings (41, 42), this functional enrichment analysis supports a role of molecular processes implicated in neuroinflammation and synaptic function in the development of epilepsy.

While these results highlight the utility of meta-analysis in increasing the value of existing preclinical data and identifying common mechanisms across different animal models, many limitations of the study should be taken into account: (1) we compared data sets that used different microarray platforms, and therefore some miRNAs may be missing in one or another; (2) miRNAs are only one of the many factors regulating gene expression, and others (histone modifications, DNA methylations, changes in transcription factors) should be taken into account; (3) this analysis focused on a specific hippocampal subarea and cell population, the DG GCs, but other brain areas and other cell populations may be at least equally important; (4) epileptogenesis and chronic epilepsy develop differently in different models, implicating that changes in miRNAs may dynamically vary at different time points; (5) anti-correlation assumes that miRNAs decrease the levels of their target mRNAs, which is the best characterized, but not the only mechanism of miRNA action (43).

This all notwithstanding, the miRNAs identified in our meta-analysis and their predicted effects on mRNAs generate hypotheses about the molecular mechanisms underlying epilepsy that will deserve further investigation. In addition, these findings may be cross-analyzed with those generated using proteomics to further corroborate the working hypotheses, in a research model in which big data obtained with different approaches are interconnected. These investigations may ultimately lead to miRNA-based therapies, which are already under preclinical investigation and may hold some advantages over traditional drug treatments in terms of precision for specific forms of epilepsy or even for specific phases of the disease. Unfortunately, the estimated costs of these therapies would be (at least for now) extremely high. Interestingly, the number and heterogeneity of the identified miRNAs suggest that therapies targeting a single

TABLE 1 | Differentially expressed microRNAs in the epileptogenesis and in the chronic period, as compared with controls.

Epileptogenesis	Chronic period
Upregulated	Downregulated
miR-21-5p	*miR-130a-3p*
miR-132-3p	miR-148b-3p
miR-146a-5p	*miR-324-3p*
miR-212-3p	miR-551b-3p
miR-212-5p	miR-652-3p
miR-344b-2-3p	
Downregulated	
miR-7a-5p	
let-7b-3p	
let-7d-3p	
miR-29c-5p	
miR-33-5p	
miR-92b-3p	
miR-101a-3p	
miR-136-3p	
miR-138-5p	
miR-139-5p	
miR-150-5p	
miR-153-3p	
miR-324-5p	
miR-330-3p	
miR-335	
miR-345-5p	
miR-383-5p	
miR-551b-3p	
miR-667-3p	
miR-3573-3p	

MicroRNAs in italics were not identified as differentially expressed in the individual studies.

miRNA may be insufficient to interfere with the epileptogenic process. This conclusion is in line with the idea of complex networks, but does not exclude the possibility of ultimately identifying sets of new potential therapeutic targets (rather than single ones).

CONCLUSION

As noticed, the studies described in this short review capture only a component of a more complex network, at a low hierarchical level. Understanding of networks not only from genetic but also from local neural and global brain data will ultimately allow identification of new targets for the treatment of seizures and epilepsy comorbidities (11).

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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The Quest for an Alzheimer Therapy

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This mini-review considers three different approaches to the therapy and prevention of Alzheimer's disease (AD): replacement therapy, disease modification, and multi-level interventions. Each of these research frameworks has direct implications at the clinical level, leading to an emphasis on different time points of the AD continuum. While all perspectives continue to play an important role in current efforts to reach the ambitious target of an effective therapy or prevention of AD by 2025, it is clear that novel paradigms are needed, including new models of clinical trial design. This goal can only be accomplished by a concerted effort of academia, governmental agencies, and industry.

Keywords: Alzheimer disease, dementia prevention, amyloid, therapy, diagnostic criteria

INTRODUCTION

The increasing awareness that dementia, and in particular Alzheimer's disease (AD), represents one of the major challenges to health systems in coming years has led to an unprecedented emphasis on the need for an effective therapy, now considered as a priority for science and society (1). The year 2025 has been set by world leaders as the target for the availability of an effective therapy or prevention of AD (2).

The modern history of research for an AD therapy can be conceptualized in three different approaches, which are briefly discussed in this review: replacement therapy, disease modification, and multi-level intervention. These approaches cannot be conceived as stages in an evolutionary process, since all of them are still playing a central role in current research. It must be, however, underlined that they have different implications for the process of diagnosing AD, as manifested by the progressive changes in diagnostic criteria, and guidelines that have taken place in the past decades, from the NINCDS-ADRDA Work Group (3), to the current research framework promoted by National Institute on Aging and Alzheimer's Association (NIA-AA) with the aim of updating and unifying the most recent published set of diagnostic criteria (4, 5).

REPLACEMENT THERAPY FOR COGNITIVE DYSFUNCTION

The successful introduction of L-DOPA for Parkinson's disease had a clear impact on the AD therapy research field. The concept that disease manifestations could be defined in terms of neurotransmitter loss was a central component of the cholinergic hypothesis, and led to the development of the leading class of drugs currently approved for AD therapy, i.e., acetylcholinesterase (AChe) inhibitor drugs. The systematic search for a biochemical fingerprint for AD began in the 1960s of past century, and was crowned by success with the discovery of reduced level of choline acetyltransferase, the enzyme responsible for ACh synthesis, in the cortex of AD patients (6), soon linked to neural loss in the nucleus basalis of Meynert (7). These findings opened the way to the search of possible "replacement" therapies, clearly modeled on the PD approach (8), and based on the prediction that drugs increasing cholinergic neurotransmission in the AD brain could be expected to result in a symptomatic treatment of the cognitive hallmark of AD, i.e., memory dysfunction. This concept was additionally supported by the evidence of a dysmnestic effect of anticholinergic drugs, such as scopolamine, in experimental

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and human studies (9). After some disappointments due to the failure of increase central ACh by direct administration of ACh precursors, the era of AChEI was opened by the tacrine trials. An excellent review of the state-of-the-art at the end of the past century can be found in Ref. (10), where the limits of the "replacement" approach are also acknowledged. Attempts to extend the neurotransmitter replacement idea to other molecules potentially associated with specific aspects of cognitive dysfunction (e.g., noradrenaline and attention) also met with limited success (11).

At the diagnostic level, this approach is necessarily linked to the identification of the presence of cognitive deficits, possibly reflecting specific neurotransmitter dysfunctions, which can become the target of a specific replacement therapy. The NINCDS-ADRDA criteria (3), which remained the standard diagnostic reference in the field of AD for more than two decades, require the presence of dementia for the definition of probable AD. The diagnostic process delineated in these criteria is purely clinical/neuropsychological, and the "ancillary examinations" are used to provide exclusionary evidence for other possible causes of the dementia syndrome.

The need for symptomatic treatments based on neurotransmitter mechanisms is not limited to cognitive enhancement, but includes the clinically crucial aspect of control of neuropsychiatric symptoms, the main determinant of burden of care in AD patients (12). In a recent review of current clinical trials in AD (13), 14% of the drugs under trial are symptomatic cognitive enhancers, and 13% are symptomatic agents addressing neuropsychiatric and behavioral changes. Most of these drugs have mechanisms of action involving different neurotransmitter systems, such as 5-HT or cannabinoids, indicating the continuing role of this approach, in particular at the level of dementia care (14).

THE HOLY GRAIL OF DISEASE MODIFICATION

The "modern era" of AD research is closely linked to the birth of the amyloid hypothesis of the pathogenesis of disease in the early 1990s. The foundations of this hypothesis were provided by the discovery of amyloid as the main component of the senile plaques and of the pathogenetic role of amyloid precursor protein (APP) mutations [see in Ref. (15) for an early review]. These findings promoted a change of focus from the condition of established dementia to the early stages of disease, which could represent a target for interventions whose ambition was the modification of the disease process, rather than a replacement of the consequences of brain damage. The search for a disease-modifying treatment (DMT) for Alzheimer's disease (AD) was motivated by "advances in the understanding of neurodegenerative mechanisms in Alzheimer's disease" in areas, such as neurotrophic factors, protein processing, oxidative stress, and inflammatory processes (16). The main focus of DMT approaches has centered on the amyloid cascade hypothesis [ACH—for an updated review, see in Ref. (17)]. Several different therapeutic approaches have been developed within this conceptual framework (**Figure 1**), which attributes a central role in neuronal degeneration and cognitive decline to the pathological aggregation of amyloid β (Aβ) peptides, predominantly $A\beta_{40}$ and $A\beta_{42}$. Aβ is derived from APP *via* proteolysis by two enzyme complexes, β-secretase and γ-secretase. While the alternative pathway, mediated by α-secretase, results in the production of a soluble sAPPα fragment, under pathological conditions the soluble oligomers of $A\beta_{42}$ result in synaptic dysfunction (decrease in synapse number, inhibition of long-term potentiation, and enhancement of long-term

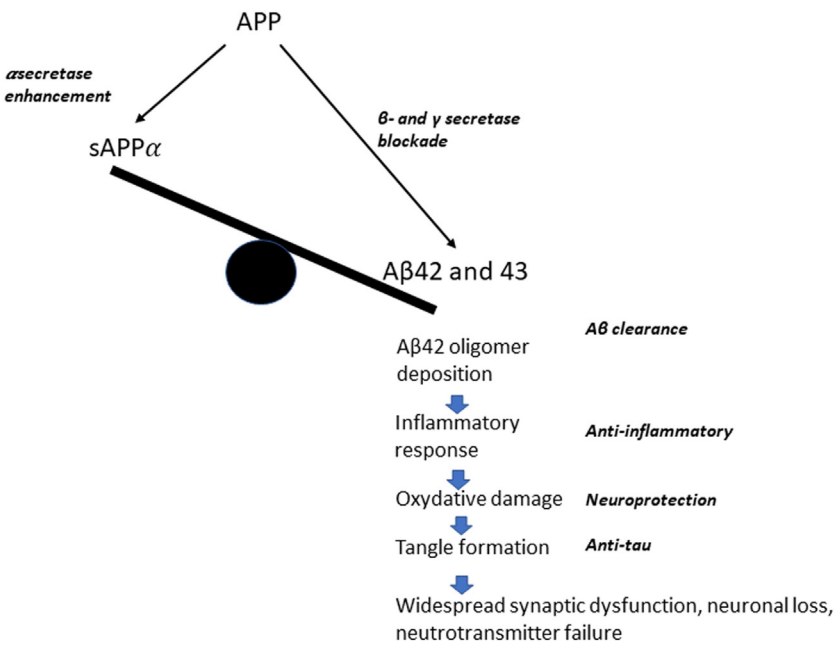

FIGURE 1 | A simplified diagram of the possible therapeutic approaches, according to the amyloid cascade hypothesis.

synaptic depression), starting a complex chain of molecular events and cellular reactions finally leading to plaque formation and neuronal death [for an updated review, see in Ref. (18)]. A model of these pathological conditions was provided by the rare dominantly inherited forms of AD, where missense mutations in the APP or presenilin 1 or 2 genes lead to a lifelong increase of $A\beta_{42}$ and $A\beta_{43}$ production, i.e., longer, more hydrophobic forms than $A\beta_{40}$, which, even in low amounts, may have higher neurotoxic potential (17). The original formulation of the amyloid cascade hypothesis proposed that "accumulation of Aβ in the brain is the primary influence driving AD pathogenesis. The rest of the disease process, including formation of neurofibrillary tangles containing tau protein, is proposed to result from an imbalance between Aβ production and Aβ clearance" (15). The possible therapeutic approaches could then be targeted toward increasing Aβ clearance or modulating its production. The first application of the hypothesis aimed at Aβ clearance, *via* an active immunization approach. The clinical trial with the AN 1792 vaccine was successful in enhancing the production of Aβ antibodies, but failed to show any clinical effect and had to be stopped because of the occurrence of serious cases of meningoencephalitis in treated patients (19). The Aβ clearance approach has remained dominant, and includes both active (vaccine) and passive (monoclonal antibodies) immunization. The latter approach has been extensively pursued in the past decade, with systemic infusion of monoclonal Abs (mAbs) aiming at preventing oligomerization and fibril formation and dissolving Aβ aggregates [for a review, see in Ref. (20)]. The failure of several large-scale trials has generated some legitimate disappointment, but has also provided important lessons about the need to carefully consider the mechanisms of action of different mAbs and the target population (see below). Several trials with newer agents, such as the fully human IgG1 mAb aducanumab (Biogen, Inc.), are ongoing and are supported by positive preliminary evidence of biological efficacy (21). An alternative approach within the same conceptual framework is based on drugs aiming at the modulation of beta amyloid production, with drugs targeting the gamma secretase complex to decrease Aβ production. The relevance of side effects has resulted in many failures to complete the studies, and has led to an increasing interest in the inhibition or modulation of beta secretase, with β-site APP cleaving enzyme 1 (BACE1) as a favorite target (20). The ACH-based therapeutic framework is completed by drugs promoting Aβ degradation (Aβ-degrading proteases) and inhibiting amyloid aggregation (chaperones) (20). A less explored approach is the activation of alpha secretase, with the rationale to stimulate the production of soluble ("good") APP, which has been attempted with a synthetic retinoid, acitretin (22).

Overall, the appraisal of the results of trials based on the ACH is at the moment negative. The so-called "tau hypothesis" is often considered as a competing approach, but it may be remarked that it shares most of the conceptual assumptions of the amyloid approach, i.e., the idea that the development of AD could be stopped or delayed by interfering with a primary pathological event, i.e., in this case the formation of neurofibrillary tangles. The development of drugs targeting tau aggregation has proven more challenging than in the case of β amyloid, and only recently drugs targeting tau aggregation and of active and passive immunization approaches have entered the clinical trial arena. Among the current clinical trials of DMT, 14 address amyloid targets and only 4 involve tau-related targets (13).

The search for a "magic bullet," which could halt or slow down the pathological process whose result is the clinical picture of dementia, has had a fundamental impact at the clinical level. Considering dementia as the irreversible outcome of a linear sequence of events unleashed by pathological protein aggregation inevitably leads to the consideration that pathophysiological process leading to dementia begins many years prior to overt clinical manifestations of disease. An early step in this direction was the definition of a "pre-dementia" stage of AD, mild cognitive impairment (23). The following steps were the quest for biomarkers, independent from the presence of clinical symptoms, sufficient to detect the presence of brain pathology. This was an important shift of focus for the diagnostic process. Since, cognitive and functional impairment could not be considered as an effective clinical endpoint for therapeutic trial, the impact of new drugs on AD pathology, the need for human disease biomarkers, derived from structural, functional, and molecular neuroimaging, as well as from neurochemical and genetic studies of AD was widely acknowledged (24). This change of perspective was instrumental in the development of new diagnostic criteria: the International Working Group Research Criteria (25–27), and the National Institute on Aging—Alzheimer's Association workgroups on diagnostic guidelines (5). The shared concept by these guidelines, i.e., the AD continuum, emphasizes the role of biomarkers in supporting the diagnosis of AD at the very early clinical stages, i.e., when the patient is symptomatic, but does not fulfill the criteria for dementia (prodromal AD, or MCI due to AD). The first step of the diagnostic process, a true gateway to the application of biomarkers, is the presence of memory impairment, defined on the basis of specific tests assessing delayed recall impairment due to medial temporal lobe dysfunction (28). The biomarkers in use aim at the *in vivo* assessment of markers of pathophysiology [amyloid positron emission tomography (PET), low cerebrospinal fluid (CSF) $A\beta_{42}$, and elevated phosphorylated tau (P-tau) and neurodegeneration (AD pattern of FDG PET hypometabolism and hippocampal atrophy on MRI)]. The series of substantial failures of drugs acting on the ACH has been largely attributed to the failure to address the disease process at the earliest possible stages, i.e., before neurodegeneration has taken place. The potential of this approach to early disease stages is now actively investigated in several clinical trials and the results will become available in the next few years.

MULTI-LEVEL INTERVENTIONS

The molecular and mechanistic views described in the previous section maintain their crucial role in the quest for a therapy of AD, and more encouraging results of the anti-amyloid (and anti-tau) approaches may be forthcoming. It is, however, now clearly acknowledged that the "neuron-centric, linear cascade initiated by Ab and leading to dementia" (18), implying direct causation, needs a revision. AD is a brain disorder, which needs to be investigated at all the multiple levels (cells, networks, computations) intervening between genes and molecules and the clinical

phenotype (29). At the molecular and cellular level, this is now acknowledged by approaches considering the complex interplay of neuronal changes with responses of astrocytes, microglia, and of the vascular compartment (18). An increased consideration of the contributing role of neuroinflammation, metabolic modifications, including stress reaction and of neuroprotective agents is testified by the presence of 25% of drugs involving these mechanisms of action under investigation in current clinical trials (2). The emphasis on a long-term chain of feedforward and feedback cellular reactions taking place during the years corresponding to the prodromal phase of AD is in full agreement with the concept of neurodegenerative disorders as progressive dysfunctions of brain-system-specific connectivity (30). While advances in neuroimaging techniques have been a major impulse toward the development of this concept (31), important complementary approaches are emerging from the field of neurophysiology, including the investigation of network-level neural activity changes, such as hypersynchrony and altered rhythmic oscillatory activity, possibly related to interneuronal dysfunction (32), in AD brain. The therapeutic implications of this approach are now starting to be considered (33). The quest for effective drugs targeting protein accumulation can be integrated in a multimodal perspective, considering different levels of intervention at the same time, including treating inflammatory reactions, modulating network dysfunction with invasive and non-invasive neurostimulation, and acting on cognitive dysfunction with lifestyle interventions and cognitive training. The latter aspect has been extensively investigated in the past decades. A recent, outstanding review by Livingston et al. (14) concluded that about 35% of dementia is attributable to a combination of nine risk factors: low educational level, midlife hypertension and obesity, hearing loss, late-life depression, diabetes, physical inactivity, smoking, and social isolation. The concept of cognitive/brain reserve is based on solid experimental evidence of protective effects on cognitive decline and dementia for physical activity, Mediterranean diet, cognitive training, and social engagement (34). The possibility to act positively on the reserve by means of active intervention is suggested by cognitive training studies in healthy elderly subjects, indicating a significant reduction of physiological cognitive decline with working memory training (35). The positive effect of training was not limited to test performance, but extended to functional activities of daily living (36). The FINGER study in at-risk subjects, based on a comprehensive intervention on risk factors combined with cognitive training, reported significant effects on several cognitive variables (37). The multi-dimensional perspective, with its focus of modification of factors increasing and decreasing the risk of cognitive decline, has an inevitable impact on the target population for intervention studies. The focus is moving from the very early clinical stages, characterized by mild symptoms, to asymptomatic at-risk subjects. Preclinical AD trials aim at finding treatments that can postpone, reduce the risk of, or completely prevent the clinical onset of AD (38, 39). The role of biomarkers at this point becomes central, as clinical variables are considered as late markers of disease progression.

CONCLUSION

Much has changed in the two decades in the quest for an effective therapy and prevention of AD. The success rate of drug development for AD has been poor and novel paradigms are needed, including new models of clinical trial design taking into account the advances in early diagnosis, the role of genetic factors, and new epidemiological evidence. This can only be accomplished by a concerted effort, including governmental agencies, academic researchers, and industry (1).

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The author confirms being the sole contributor of this work and approved it for publication.

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Centrality of Early Synaptopathy in Parkinson's Disease

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Significant advances have been made in the understanding of the numerous mechanisms involved in Parkinson's disease (PD) pathogenesis. The identification of PD pathogenic mutations and the use of different animal models have contributed to better elucidate the processes underlying the disease. Here, we report a brief survey of some relevant cellular mechanisms, including autophagic–lysosomal dysfunction, endoplasmic reticulum stress, and mitochondrial impairment, with the main aim to focus on their potential convergent roles in determining early alterations at the synaptic level, mainly consisting in a decrease in dopamine release at nigrostriatal terminals and loss of synaptic plasticity at corticostriatal synapses. In a number of experimental models, this synaptopathy has been shown to be an initial, central event in PD pathogenesis, preceding neuronal damage, thereby representing a valuable tool for testing potential disease-modifying treatments.

Keywords: Parkinson's disease, cellular mechanisms, synaptopathy, dopamine transmission, animal models

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INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disorder, currently affecting 1% of the population above 60 years (1) and characterized by progressive motor deficits, including akinesia (or bradykinesia), rigidity, resting tremor, and postural instability (2). The neuropathological hallmarks of PD are the progressive loss of substantia nigra pars compacta (SNpc) dopaminergic neurons (DAns) and the presence of intraneuronal α-synuclein cytoplasmic inclusions, termed Lewy bodies (3). In the past two decades, a number of pathogenic mutations associated with PD have been identified (4), improving our understanding of pathogenic disease mechanisms. Many PD-related genes, such as *SNCA*, *PINK1*, *GBA1*, have a crucial role in different cellular mechanisms that have proven to be involved in PD, including autophagy/lysosome pathway, endoplasmic reticulum (ER) stress, and mitochondrial impairment (5). In this regard, the purpose of this review is to provide a brief overview of the most relevant pathogenic mechanisms, but with a specific focus on clues supporting early synaptic dysfunction as a functional and structural event that could represent a final convergent phenomenon for multiple distinct processes. Comprehensive review of PD-related pathogenic mechanisms is beyond the scope of this survey, and we refer the readers to other recent excellent reviews (5, 6).

SYNAPTOPATHY IN PD

Despite considerable progress in our understanding of the aberrant mechanisms involved in PD pathogenesis, some key questions remain unanswered. Among these, a central issue is to establish the precise sequence of events at the cellular level and where the pathogenic process begins. Multiple lines of evidence suggest that the primary site of α-synucleinopathy is represented by the synaptic

terminal, with the occurrence of an early synaptic impairment that precedes axon degeneration and with subsequent retrograde progression through a dying-back mechanism (7) (**Figure 1**). This "synaptopathy" described at the cellular level could correspond to an early, presymptomatic time window in patients, when only a 30–50% decrease in striatal dopamine levels can be detected, providing evidence for a powerful ability of the motor system to compensate (8). The concept of synaptopathy is closely related to α-synuclein, the major constituent of Lewy body, which, in physiological conditions, is primarily localized to the presynaptic terminals, where it affects the fusion and clustering of synaptic vesicles, thus influencing neurotransmitter release (9–11). Evidence on how α-synuclein plays a crucial role in synaptic function and plasticity comes from several studies on animal models. In a transgenic mouse model of α-synucleinopathy (αSyn 1-120 mice), α-synuclein aggregates were detected at striatal dopaminergic terminals, with an impairment of dopamine release from nigrostriatal synaptic terminals, even in the absence of nigral DAn loss (12). In another model, a bacterial artificial chromosome (BAC) transgenic mouse with overexpression of human wild-type α-synuclein (SNCA-OVX), a clear time-dependent progression was observed: in 3-month-old mice, in spite of the absence of overt neuropathology, early deficits in dopamine release in the dorsal striatum and increased clustering of vesicles in dopamine terminals were found (13). Conversely, at 18 months, mice showed motor deficits, loss of dopamine neurons, and a reduced firing rate in the remaining SNpc dopamine neurons, further indicating synaptic dysfunction as an early event. Of relevance, this feature does not apply only to α-synuclein models. Many other PD animal models, including those based on the administration of the "classical" neurotoxins 6-OHDA and MPTP, and the ones with genetic mutations not involving α-synuclein, have contributed to identify synaptic dysfunctions occurring at early stages of the disease. In a 6-OHDA model of early PD with partial denervation and mild motor alterations, the decreased level of dopamine observed was responsible for a selective impairment of corticostriatal synaptic plasticity recorded from spiny projection neurons (SPNs), with a specific deficit of long-term potentiation (LTP) and with sparing of long-term depression (LTD) (14). Similar corticostriatal synaptic plasticity impairments were also found by Chou et al. (15), who performed electrophysiological recordings from SPNs and from SNpc dopaminergic cells of 8–9-month-old *LRRK2* (G2019S mutation) transgenic mice. *LRRK2* is a multidomain protein with kinase activity, whose mutations are involved in autosomal dominant forms of PD (16). The function of LRRK2 has not been fully elucidated, although strong evidence implicates a role in intracellular trafficking, vesicular recycling, and modulation of synaptic transmission (17). In line with this, Chou et al. identified an early decrease in spontaneous firing frequency of SNpc dopaminergic cells, without gross degeneration of nigrostriatal terminals, and impaired evoked dopamine release, with subsequent deficit in LTD induction in striatal neurons (15). Moreover, a recent study on *LRRK2* BAC transgenic rats revealed alterations to dopamine circuit function, in the form of L-DOPA-responsive motor dysfunction, a reduction in SNpc dopamine neurons burst firing, and an impaired striatal dopamine release, occurring in the absence of neurodegeneration or abnormal protein accumulation (18). Besides the examples reported so far, the concept of synaptopathy can be extended to various PD models and appears to be linked to different cellular mechanisms, as discussed in the following sections.

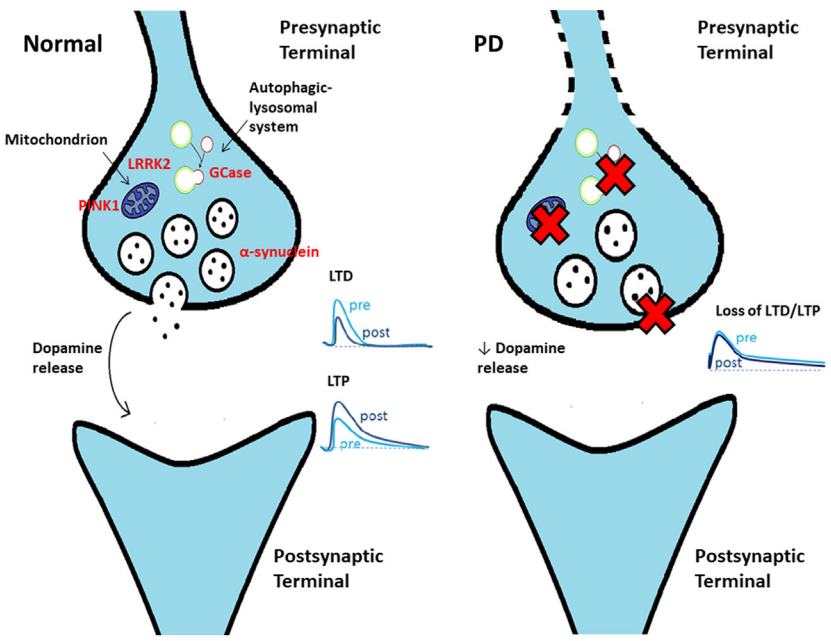

FIGURE 1 | Schematic cartoon showing a synaptic terminal in control conditions (left) and in Parkinson's disease (PD) (right). Several mechanisms contribute to early dysfunction at the terminal level, causing an impaired dopamine release. As a consequence, synaptic plasticity at corticostriatal synapses [long-term depression (LTD) and long-term potentiation (LTP)] is impaired in PD.

DYSFUNCTIONAL AUTOPHAGY–LYSOSOME SYSTEM AND THE ROLE OF *GBA1* MUTATIONS

Autophagy, acting through lysosomal degradation, represents the main proteolytic system in neurons (19). An abnormal autophagic activity leads to the accumulation of aberrant proteins and toxic components (20), contributing to the neurodegeneration observed in several diseases, including PD (21). In PD, dysfunctional autophagy is responsible for the accumulation of α-synuclein: specifically, two types of autophagy (macroautophagy and chaperone-mediated autophagy), both involved in α-synuclein degradation, appear to be impaired in PD (22). In addition, growing evidence indicates that excessive α-synuclein itself blocks these degradation pathways, promoting α-synuclein aggregation (23). Experimental findings show that Lewy body-like aggregates are able to resist to macroautophagy degradation by impairing clearance of autophagosomes (24) and that two α-synuclein familial mutations, *SNCA-A30P* and *SNCA-A53T*, can alter the chaperone-mediated autophagy pathway (25). In the recent past, a growing body of evidence suggests a prominent role for decreased glucocerebrosidase (GCase) activity in autophagic failure and subsequent α-synuclein accumulation in PD (26). GCase is a lysosomal enzyme encoded by the *GBA1* gene, ubiquitously expressed in the brain with some regional variations (27), whose homozygous or heterozygous compound mutations cause Gaucher disease (GD), with the accumulation of glycolipid substrate (28). Of note, heterozygous *GBA1* mutations represent the most relevant risk factor for PD, since they can be found in approximately 5–10% of idiopathic PD patients (29). The majority of *GBA1* mutations are associated with reduced GCase levels (30), with milder mutations responsible for slightly diminished enzyme levels, conferring a much lower risk of PD than mutations causing severe enzymatic dysfunction (31). The molecular mechanisms underlying the increased PD risk in *GBA1* mutation carriers have not been fully clarified. A dual interplay has been proposed for GCase and α-synuclein. On the one hand, GCase loss-of-function would lead to aberrant lysosomal protein degradation and neurotoxicity, whereas on the other hand α-synuclein may inhibit the activity of normal GCase (32). Accordingly, in postmortem samples of PD patients without *GBA1* mutations, a reduced GCase activity has been reported (33). The scenario is even more complex, considering that significant loss of GCase activity can cause neurodegeneration even in the absence of α-synuclein (34). To better elucidate the precise mechanisms linking *GBA1* with parkinsonism, a number of disease models have been developed, including both animal models and cell-based models. *GBA1* knockout mice and transgenic mouse lines carrying *GBA1* point mutations well recapitulate GD phenotype, with accumulation of α-synuclein and ubiquitinated proteins, the presence of typical "Gaucher cells" and inflammation (28), but they also prove to be appropriate for the study of the effects of GCase reduction in PD pathogenesis. In the knockout mice, for instance, the lysosomal defect demonstrated in neurons and astrocytes lacking *GBA1* was correlated with dysfunctional and fragmented mitochondria, pointing out a possible relationship between decreased GCase activity and impaired mitophagy, due to the inhibition of the degradation of mitochondria by the autophagy–lysosome pathway (35). However, it is important to note that *GBA1* mutations do not act only through defective lysosome pathway: on the contrary, GCase loss-of-function may be associated with multiple other pathogenic cellular mechanisms, including ER stress, calcium metabolism dysregulation, and neuroinflammation (36), that, altogether, might contribute to the aggregation of misfolded α-synuclein. In this context, any disease-modifying therapy designed to increase GCase levels would act at different pathogenic levels, with the final target to slow down the progressive aggregation of α-synuclein (37, 38). In view of the close linkage between GCase function and α-synuclein deposition, it could be questioned whether this lysosomal enzyme deficiency can also affect synaptic function. A relatively recent experimental study gives interesting insights about this issue: in a murine model of PD, where a subchronic conduritol-β-epoxide exposure induced GCase inhibition, Ginns and colleagues identified an early synaptic impairment, in the form of a reduction of striatal evoked dopamine release and altered synaptic plasticity markers, including post-synaptic density size and miRNA expression levels, together with glial activation within nigrostriatal pathway and abnormal α-synuclein accumulation (39). The effects of *GBA1* insufficiency on dopaminergic neurotransmission and synaptic function documented in this animal model fit with clinical observations. Indeed, patients carrying *GBA1* mutations show early striatal presynaptic dopaminergic dysfunction even before the onset of motor symptoms (40, 41). Further studies of individuals carrying a mutant *GBA1* allele, together with the development and characterization of different *GBA1* models, will help to clarify the mechanisms underlying the Parkinson's–GD connection and to provide novel insights into the influence of diminished GCase activity on synaptic transmission, which could lead to develop novel therapeutic interventions.

ER STRESS

The ER represents a quality control system to check the correct protein folding, while misfolded or unfolded proteins are directed toward cytosol for the degradation by ER-associated degradation system (42). The accumulation of misfolded proteins inside the ER lumen, defined ER stress, is a toxic process to which the cell reacts by activating the unfolded protein response (UPR) (43), with the aim to restore ER homeostasis. Conversely, if the adaptive response is insufficient, the cell undergoes apoptosis. The involvement of ER stress has been demonstrated in several neurodegenerative conditions, including PD (44), and many reports prove the role of some PD-related genes in this cellular process. For example, differentiated PC12 cells with expression of A53T mutant α-synuclein show decreased proteasome activity and increased ER stress (45). Moreover, a recent study performed on iPSC-derived DAns carrying GBA-N370S mutation demonstrates the activation of UPR with upregulation of ER-resident chaperones (46). Given the existence of an interplay between mitochondrial and ER stress (47), also mitochondrial proteins such as Parkin and PTEN-induced putative kinase 1

(PINK1) have a crucial role in this mechanism. Activation of ER stress, mediated by mitofusin bridges occurring between defective mitochondria and the ER, has been reported in Drosophila PINK1 and Parkin mutants (48). Despite the growing interest in this field, the contribution of ER stress to neuronal death still needs further investigation, in view of a potential application in PD therapeutics (49). In this regard, interesting inputs come from a very recent study performed on three different rodent models of PD, in which the authors described an activation of RNA-like ER kinase (PERK) signaling, as well as in postmortem brain tissue derived from parkinsonian patients (50). PERK is a crucial ER stress sensor, whose chronic signaling blocks the translation of essential synaptic proteins, impacting neuronal survival and synaptic function. In these experimental settings, PERK inhibition exerted a neuroprotective effect, as evidenced by an increase in dopamine levels and in the expression of synaptic proteins. This once again highlights the relevance of synaptopathy in multiple aberrant mechanisms in PD.

MITOCHONDRIAL IMPAIRMENT

Most neurodegenerative diseases share a mitochondrial impairment as a major pathophysiological hallmark. Mitochondria are the main source of chemical energy for the cell and, as a consequence, their dysfunction leads to decreased levels of ATP and production of reactive oxygen species, which negatively impact neuronal physiology and, ultimately, cell survival (49). An impaired mitochondrial complex I activity has been demonstrated in the SNpc of PD patients (51). It is well established that many environmental toxins act as potent mitochondrial complex I inhibitors, and accordingly, different toxin-based experimental models of PD have been developed in order to reproduce mitochondrial dysfunction. MPTP, acting through its metabolite MPP^+, causes loss of dopaminergic SNpc neurons, inducing parkinsonian features in mice and non-human primates (52). The same toxin has been used to develop MPTP-treated models with only partial dopaminergic deafferentation, in which an early reduction in spine density in both the caudate nucleus and putamen could be detected, as an early pathological hallmark of the disease (53). Rotenone, a largely used pesticide, is another mitochondrial complex I toxin, and its administration reproduces many histochemical and behavioral features of human PD in rodents and non-human primates, including selective nigrostriatal dopaminergic lesions and α-synuclein-positive cytoplasmic aggregates in nigral neurons (54). Such experimental evidence has been further confirmed by a number of epidemiological studies (55). Mitochondrial dysfunction and oxidative stress are tightly connected to PINK1, a serine/

TABLE 1 | Synaptopathy in different animal models of Parkinson's disease.

Animal model	Model generation	Motor behavior	Nigral dopaminergic neuron loss	Synaptic alterations	Reference
α-syn (1-120) transgenic mice	Expression of truncated human α-syn (1-120)	Reduced locomotion	NO	Age-dependent reduction in dopamine release	(12)
BAC transgenic mice (SNCA-OVX)	Overexpression of human wild-type α-syn	Normal (3 mo of age) Motor deficits (18 mo of age)	No (3 mo of age) Yes (18 mo of age)	Reduced firing rate of SNpc dopamine neurons (18 mo of age) Increased clustering of vesicles in dopamine terminals Deficit in dopamine release	(13)
Unilateral 6-OHDA rat model	Partial dopamine denervation	Mild motor alterations	Partial	Selective impairment of corticostriatal LTP with sparing of LTD	(14)
8- to 9-month-old *LRRK2* transgenic mice	Expression of G2019S mutant *LRRK2*	Hypoactivity	NO	Reduced firing rate of SNpc dopamine neurons Impaired evoked dopamine release Impairment of corticostriatal LTD	(15)
LRRK2 BAC transgenic rats	Expression of G2019S or R1441C mutant *LRRK2*	L-DOPA-responsive motor dysfunction	NO	Reduced burst firing of SNpc dopamine neurons (R1441C rats) Impaired dopamine release	(18)
CBE mouse model	Subchronic CBE exposure to inhibit GCase	Motor impairments	Glial activation in nigrostriatal pathway	Reduced evoked striatal dopamine release Altered synaptic plasticity markers	(39)
Unilateral 6-OHDA mouse model	Dopamine denervation Evidence of activation of PERK signaling	Motor impairments Attenuation of motor deficits after PERK inhibition	YES Reduced neuron loss after PERK inhibition	Lower levels of striatal dopamine, with complete recovery after PERK inhibition Reduced expression of synaptic proteins (VAMP2 and SNAP25), partially reverted after PERK inhibition	(50)
PINK1$^{+/-}$ mice	Heterozygous *PINK1* knockout mice	Normal	NO	Lower striatal dopamine release Selective impairment of corticostriatal LTP with sparing of LTD	(58)

The table summarizes early synaptic impairments reported in different PD models.
α-syn, α-synuclein; BAC, bacterial artificial chromosome; SNpc, substantia nigra pars compacta;
LTD, long-term depression; LTP, long-term potentiation; CBE, conduritol-β-epoxide; GCase, glucocerebrosidase; mo, months.

threonine kinase located in the intermembrane mitochondrial space and involved in the mitophagic pathway (56). *PINK1* loss-of-function mutations are linked to inherited early-onset forms of PD (57). In a mouse model carrying heterozygous PINK1 mutations, we identified early rearrangement within corticostriatal circuitry, expressed by selective impairment of LTP with a physiologically expressed LTD, in the absence of motor phenotype and dopaminergic neuronal loss (58). These observations were in line with the results of neuroimaging and physiological studies performed on *PINK1* heterozygous mutation carriers manifesting initial alterations in the nigrostriatal circuit (59, 60). Accordingly, the heterozygous condition related to familial parkinsonism represents an ideal preclinical model, which allows us to study early alterations occurring before the onset of motor signs, in a time window suitable to test potential novel disease modifying therapy (61). A number of attempts have been made to recreate the gene–environment interaction that might underlie disease pathogenesis. Recently, we exposed *PINK1* heterozygous knockout mice to rotenone, which was chronically administered at very low doses. Of interest, combination of gene mutation with minimal rotenone exposure was able to cause severe alterations of corticostriatal synaptic plasticity, to an extent similar to that observed in the *PINK1* homozygous knockout model (62, 63). The experimental use of toxins inducing mitochondrial impairment has contributed over the past decades to improve our knowledge on the pathogenic mechanisms of neurodegenerative diseases, in an attempt to develop neuroprotective agents and etiologic treatments.

CONCLUSION AND FUTURE DIRECTIONS

Over the past decades, major advances have been made in the understanding of the mechanisms involved in PD pathogenesis and the mechanisms that, furthermore, share common elements and contribute synergistically to neuronal dysfunction. Growing evidence attributes an undisputed central role to α-synuclein aggregation, also in view of its interaction with multiple processes, including intracellular trafficking, mitochondrial dysfunction, ER stress, and lysosomal dysfunction. However, there remain many unclear issues. The captivating prion-like hypothesis, according to which aggregated α-synuclein is trans-synaptically spread through the brain connectome, is still debated, as it does not fully recapitulate PD pathogenesis (64). Indeed, the pattern of spreading of Lewy-body pathology does not precisely match Braak's theory, according to a number of studies examining postmortem PD samples (65). Yet, at cellular level, the pattern of distribution of α-synuclein aggregates appears to spare brainstem GABAergic neurons (66). Such evidence highlights some limitations to the "prion-like" theory and supports the need for a more comprehensive hypothesis that could take into consideration the selective neuronal susceptibility (64). In this complex scenario, many experimental models point toward the synapse as the primary site of PD pathology and considering synapse failure as a putative common denominator. Indeed, synaptopathy is an early event in PD pathogenesis in most phenotypic and genetic models reported so far (**Table 1**). Impairment of synaptic activity and plasticity at corticostriatal synapses represents a peculiar endophenotype, in distinct models of human movement disorders (67–69). In addition, these alterations have been shown to parallel time-dependent progression of cellular demise, thereby mimicking a very important disease stage, where potential disease-modifying treatments could be tested. Understanding the molecular events leading to synaptic dysfunction, achieved by the use of suitable PD animal models, will encourage the development of potential synapse-target therapies, in the hope of actively intervene on one of the mechanisms leading to PD pathogenesis.

AUTHOR CONTRIBUTIONS

PI and AP designed the study and wrote the paper. TS and MM prepared illustrations and revised the text. NM revised the text.

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