Original Contribution

Fluoxetine Maintains a State of Heightened Responsiveness to Motor Training Early After Stroke in a Mouse Model

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Background and Purpose—Data from both humans and animal models suggest that most recovery from motor impairment after stroke occurs in a sensitive period that lasts only weeks and is mediated, in part, by an increased responsiveness to training. Here, we used a mouse model of focal cortical stroke to test 2 hypotheses. First, we investigated whether responsiveness to training decreases over time after stroke. Second, we tested whether fluoxetine, which can influence synaptic plasticity and stroke recovery, can prolong the period over which large training-related gains can be elicited after stroke.

Methods—Mice were trained to perform a skilled prehension task to an asymptotic level of performance after which they underwent stroke induction in the caudal forelimb area. The mice were then retrained after a 1- or 7-day delay with and without fluoxetine.

Results—Recovery of prehension after a caudal forelimb area stroke was complete if training was initiated 1 day after stroke but incomplete if it was delayed by 7 days. In contrast, if fluoxetine was administered at 24 hours after stroke, then complete recovery of prehension was observed even with the 7-day training delay. Fluoxetine seemed to mediate its beneficial effect by reducing inhibitory interneuron expression in intact premotor cortex rather than through effects on infarct volume or cell death.

Conclusions—There is a gradient of diminishing responsiveness to motor training over the first week after stroke. Fluoxetine can overcome this gradient and maintain maximal levels of responsiveness to training even 7 days after stroke. **(*Stroke*. 2015;46:00-00. DOI: 10.1161/STROKEAHA.115.010471.)**

Key Words: fluoxetine ■ motor cortex ■ neuronal plasticity ■ recovery ■ stroke ■ upper extremity

The largest amount of motor recovery at both the impairment and functional levels occurs in the first 4 weeks after ischemic stroke both in humans[1–4] and in rat models.[5–8] We have previously referred to this period of spontaneous recovery and increased responsiveness to motor training as the poststroke sensitive period.[9] The sensitive period is a unique, time-limited environment of heightened plasticity characterized by molecular,[8,10] physiological,[11,12] and structural changes,[13,14] which are qualitatively and quantitatively distinct to plasticity mechanisms in the absence of stroke or in the presence of a chronic stroke.[6,9] That there is a causal link between the unique short-lived plasticity milieu after stroke and the amount of recovery from hemiparesis in this same period is supported by rodent experiments that have manipulated plasticity in the sensitive period, for example, by increasing[15,16] or decreasing brain derived neurotrophic factor,[17] which augments or prevents recovery, respectively. Here, we asked whether there might be a way to augment or prolong the sensitive period. Specifically, is it possible to initiate training later after stroke but still maintain maximal responsiveness? This has great clinical relevance for those patients too medically ill to start intense rehabilitation immediately.

In a recent influential randomized placebo-controlled clinical trial, the selective serotonin reuptake inhibitor (SSRI), fluoxetine, was given within 10 days after stroke and then continued for 3 months.[18] At 3 months, the group receiving fluoxetine had significantly enhanced recovery from motor impairment when compared with the control group.[18–20] A recent meta-analysis showed that poststroke patients treated with SSRIs were less likely to be dependent, disabled, or neurologically impaired.[21] The exact mechanism of how fluoxetine potentiates recovery is not known but is likely related to its ability to augment synaptic plasticity. For example, fluoxetine can restore time-dependent visual cortical plasticity in the adult rodent,[22] likely through a decrease in the inhibition/

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excitation ratio,[22,23] and by stimulating gene expression important for plasticity.[22,24]

Using a mouse model of focal motor stroke and a skilled prehension (reach-to-grasp) task,[25] we first sought to demonstrate that there is diminished responsiveness to training when it is delayed by a week when compared with when it is initiated 24 hours after stroke. We then tested the hypothesis that fluoxetine, when administered 24 hours after stroke, would maintain a maximal response to training even after a delay of a week between stroke induction and training.

Materials and Methods

Mice

Adult male C57bl/6 mice 100 to 140 days old were singly housed in custom-made chambers and kept on a 12/12-hour light/dark cycle. A total of 70 mice were used; 2 animals were excluded from the analysis because of death before completion of the study (1 received saline and 1 received fluoxetine). The specific number of animals for each behavioral experiment was chosen based on previously published experiments,[5,25,26] and *n* is reported in each figure legend. Three to 4 days before training on the prehension task, the mice were placed on scheduled administration of daily 2.0 to 3.0 g Bio-Serv dustless precision pellet mouse chow with water ad libitum, with food restriction to 85% of their starting weight. All animal handling and use was performed according to and with approval from the Johns Hopkins University Animal Care and Use Committee.

Skilled Prehension Task

Training was conducted in an identical manner to that described before.[25] Briefly, mice were trained in modified cages to reach for 45-mg dustless precision pellets (Bio-Serv). Prehension was scored as successful when the mouse reached its forelimb through the slit, grabbed the pellet, and ate it without knocking it from its resting space, dropping it, or in any other way losing control. The percent of successful prehension attempts was determined per pellet. A training block consisted of 30 pellets at a distance of 1 cm with each pellet presented one at a time. After familiarization and paw determination, the animals underwent 2 blocks of 30 reaching attempts per training day. The animals had 1 training day off per week (including the day after stroke induction). Mice not undergoing training (days off) were treated just like the trained mice (allowed to run free in their home cage, were food restricted, fed the same pellets, and maintained on the same light/dark cycle) but were never exposed to the prehension task. Training began after a 1- or 7-day delay after stroke induction. All investigators were blinded to training condition after stroke induction.

Fluoxetine

Mice were randomized after stroke induction to receive either fluoxetine (Tocris; prepared in sterile normal saline) 10 mg/kg daily or normal saline 5 mL/kg daily via intraperitoneal injection beginning either 24 hours or 7 days after stroke induction, ie, the injections were always given 24 hours before the commencement of training. Investigators were blinded to stroke versus sham condition, as well as to fluoxetine versus saline injection.

Stroke Induction

The location of motor areas was identified based on previous anatomic[27] and functional[28] data. These data also indicate that these areas are geographically consistent within a given strain. We have used these with previous success and followed our previously published protocol.[25] A fiber optic bundle of a cold light source (Zeiss 1500 Electronic, Jena, Germany) with a 20-gauge aperture was centered at 2-mm lateral and 0.5-mm anterior from bregma for caudal forelimb area (CFA) infarction. The brains were then illuminated through the intact skull for 15 minutes, starting 5 minutes after the intraperitoneal injection of 150 µL of a 10-mg/mL rose Bengal solution in sterile normal saline. Animals undergoing sham had an identical procedure performed except that no illumination occurred.

Tissue Preparation and Histology

On the day of euthanasia, the mice were placed under deep anesthesia with 2.5% avertin and transcardially perfused with 4% paraformaldehyde in 0.1 M sodium phosphate, pH 7.4. The brains were dissected out and placed in 4% paraformaldehyde for 24 hours. Brains were coronally sliced at 50 µm on a vibrating microtome. Free-floating sections were washed 3× for 5 minutes in PBS and subsequently stained in 1 of 3 different ways.

1. Cresyl violet staining: slices were stained in 0.1% Cresyl violet with 0.25% glacial acetic acid followed by dehydration in graded alcohols. Slices were mounted in Permount (Fischer).
2. Immunofluorescent preparation: slices were placed for 4 hours in block solution (10% normal goat serum and 0.04% triton X-100, in tris buffered saline) followed by overnight incubation at 4°C with primary antibody diluted in block solution. Sigma primary antibodies diluted at 1:1000 included anticalbindin (monoclonal goat antimouse), anticalretinin (polyclonal goat antirabbit), and antiparvalbumin (monoclonal goat antimouse). Sections were subsequently washed 3× for 5 minutes in tris buffered saline with 0.04% triton X-100 and incubated at room temperature for 4 hours with secondary antibodies (Alexa goat-antirabbit 633 diluted 1:500, and goat-antimouse 488 diluted 1:250). Sections were washed 2× for 5 minutes in tris buffered saline+triton X-100, 1× for 5 minutes in tris buffered saline, and mounted in ProLong Gold reagent (Invitrogen).
3. Fluoro-Jade C preparation (Histo-Chem Inc): sections were mounted onto superfrost slides (Fischer) and allowed to dry overnight. Sections were the treated per Histo-Chem Inc protocol.

Quantification of Stroke Volume

From brain slices prepared with Cresyl violet at the indicated times of euthanasia, the entire anterior-posterior extent of the CFA contralateral to the preferred paw was imaged and reconstructed in 3 dimensions using Imaris (Bitplane) imaging software. An investigator blinded to conditions demarcated the stroke pathology, and volumes were calculated using Imaris (Bitplane) imaging software.

Inhibitory Interneuron Counts

Using coronal sections, we defined counting areas anatomically based on previous definitions of the boundaries of medial agranular cortex (AGm) [25,28] and medial frontal cortex (mFC).[27,29] For AGm, we defined a medial boundary from which we extended a 1.2-mm^2 area slice from the medial and dorsal pial boundaries; for mFC, we extended a 1.2-mm^2 area slice from the midline pial boundary. These areas represent subareas of AGm and mFC, which prevented us from confounding our counts with cells from neighboring areas. The entire extent of a 50-µm slice was then imaged at 2-µm intervals using Zeiss Apotome technology to precisely localize cells,[30] and the resulting data were reconstructed in 3 dimensions using Imaris (Bitplane) imaging software to create a 60-mm^3 volume obtained in an unbiased manner (Figure I in the online-only Data Supplement). Such volumes were taken from each animal both ipsilesional and contralesional to the stroke. An investigator blinded to the experimental condition counted the number of cells immunofluorescently labeled with parvalbumin, calretinin, or calbindin within this volume. A cell was counted as positive if it had any immunofluorescent label for the indicated marker.

Analysis of Cell Death

Seven days after CFA stroke induction, tissue was prepared for Fluoro-Jade C analysis as described above. The entire anterior-posterior extent of the CFA stroke was analyzed and sections representing the largest medial-lateral area were imaged (thus representing the central core and largest area of stroke damage). The entire extent of 50-µm slices were then imaged at 2-µm intervals using Zeiss Apotome technology to precisely localize puncta,[30] and the resulting data were reconstructed in 3 dimensions using Imaris (Bitplane) imaging software. An investigator blinded to the experimental condition counted the number of Fluoro-Jade C positive cells. A cell was counted as positive if it had any immunofluorescent label for Fluoro-Jade C. The volume of the stroke was used to normalize the Fluoro-Jade C counts.

Statistics

Behavioral data were analyzed with both generalized and linear mixed-effect models.[31,32] Separate models were fit before and after stroke induction. The basic linear model was $Y_{it} = \beta_0 + U_i + \beta_1 G_i + \beta_2 t + \beta_3 + G_i + t + \varepsilon_i$, where the Y_{it} is the percentage of correct grabs for mouse i on day t, G_i is group status (fluoxetine versus saline), U_i is a mouse-specific random intercept. These are assumed to be independent and identically distributed Gaussian random variables and ε_i are normally distributed random errors. Both natural scale and logit transformed versions of the model were considered. In addition to this model we considered a generalized linear mixed model $C_{it} \mid U_i \sim \text{Binomial}(p_{it}, n_i)$, where C_i was the count of the correct grabs for mouse i on day t out of n_i trials. The model assumed that $\log it(p_{it}) = \gamma 0 + U_i + \gamma_1 G_i + \gamma_2 t + \gamma_3 \times G_i \times t$, where $\log it(p_{it})$ is the natural logarithm of the odds of a correct reach for mouse i on day t. In both models, the random intercept and intercept terms account for any mouse- and group-specific differences at either baseline or after training. Because the results were in agreement, we report the results of the simpler linear mixed-effect model with no transformations.

Stroke volume data were analyzed using GraphPad Prism 2-way ANOVA with correction for multiple comparisons. Immunofluorescent data were analyzed using GraphPad Prism 2-way ANOVA with Tukey post-test. Cell death data were analyzed using GraphPad Prism 2-way *t* test (not assuming a Gaussian distribution).

Results

Recovery of Prehension Was Incomplete When Training Was Delayed

In a previous study, we showed that focal stroke in CFA, contralateral to the preferred paw, led to a large decrement in skilled prehension, which recovered to normal prestroke levels if training was initiated after a 1-day delay and continued for 7 days.[25] Here, as a control, we reproduced this result in mice receiving daily saline injections (the fluoxetine results will be given in the next section). Specifically, wild-type mice were trained to perform the skilled prehension task and reached asymptotic performance after 6 to 8 training days. An approximate 0.25-mm^3 focal stroke in CFA contralateral to the preferred paw led to a large decrement in prehension accuracy, which recovered fully to prestroke levels when training was started after a 1-day delay (Figure 1A).

To examine the effect a training delay would have on motor recovery, we trained wild-type mice to perform the skilled prehension task, induced a focal CFA infarction, and then had the mice remain in their home-cages for 7 days, free to move about but without prehension training. Assessment on the prehension task on poststroke day 8 revealed that there was little spontaneous recovery of performance (Figure 1B). Starting training on the prehension task on poststroke day 8 did lead to performance gains, but these were markedly lower than for mice that began training after only a 1-day delay (Figure 1A).[25] Mice that underwent sham procedures had the same performance level before and after the delay, which indicates that the 7-day delay itself did not lead to a decrement in performance via forgetting. To summarize, there was a return to normal prestroke performance on the prehension task if training was started within 48 hours, but this was not the case if initiation of training was delayed by a week; the mice improved by a small amount then hit a plateau.

Fluoxetine Increased Responsiveness to Delayed Training

The main hypothesis of this study was that fluoxetine would improve motor recovery by maintaining the poststroke sensitive period. To test this, we administered daily intraperitoneal injections of fluoxetine beginning 24 hours after CFA stroke. The experiments with fluoxetine duplicated those described above with saline. The group that began training after a 1-day poststroke delay showed a return to prestroke levels of prehension, as was seen in the analogous saline group (Figure 2A). The critical difference between saline and fluoxetine groups became apparent when training was delayed by a week: only the fluoxetine group showed a return to prestroke levels of prehension (Figure 2B). Specifically, before stroke induction, there was an estimated 3.4% point increase per day in correct reaches (SE, 0.42 with a highly significant P=1e−13). The saline group was an estimated 0.7% points lower at baseline (nonsignificant P=0.839), but there was no significant

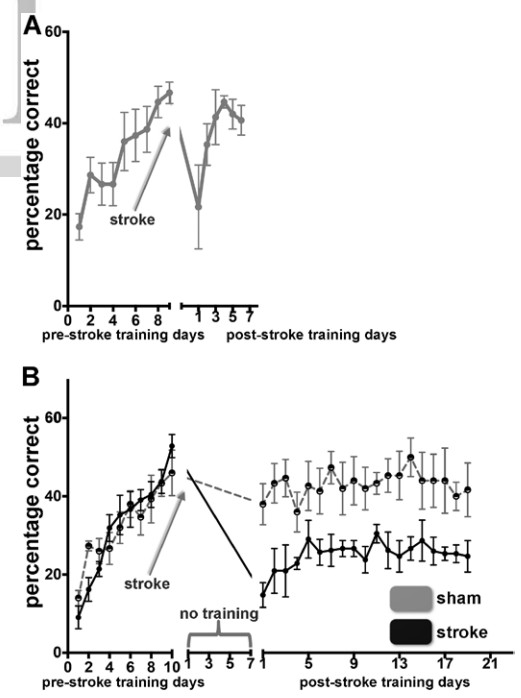

Figure 1. There is a sensitive period for motor training after caudal forelimb area (CFA) stroke. Plots show reaching success (mean±SEM). **A**, Skilled prehension completely recovered after CFA infarction when training was begun after a 1-day poststroke delay (saline administered at 24 hours; n=5). **B**, Skilled prehension recovered minimally when training was delayed by 7-days (sham stroke: gray, n = 5; stroke: black, n=7).

difference in the slope between the saline and the fluoxetine groups (P=0.6). After stroke induction, the saline group started an estimated 2% points lower on correct reaches (P=0.6). The fluoxetine group gained an estimated 4.5% points of correct reaches per day, whereas the saline group gained an estimated 1.7 per day. The estimated difference in slopes (2.8% points per day) was highly statistically significant (SE, 0.97; P=0.005).

To test whether the timing of fluoxetine administration itself has an effect on recovery, we performed the same 7-day poststroke delay experiment but with the difference that fluoxetine was only started on day 7 post stroke (Figure 2C). The learning effect before stroke was a little smaller for the fluoxetine group (1.77 increase in percent correct per day versus 2.41 increase per day for saline group). After stroke, the fluoxetine group learned at a rate of 0.98% points per day, whereas the saline group learned at 0.732 (P=0.60 for the comparison in the groups). Thus, the mice that received fluoxetine 7 days after stroke never returned to prestroke levels of prehension and looked similar to the saline group.

In summary, if fluoxetine was given 24 hours after stroke, then recovery occurred to prestroke levels if training was begun either after a 1- or a 7-day poststroke delay. This apparent fluoxetine-induced maintenance of maximal responsiveness to training over a delay only occurred if the drug was administered early.

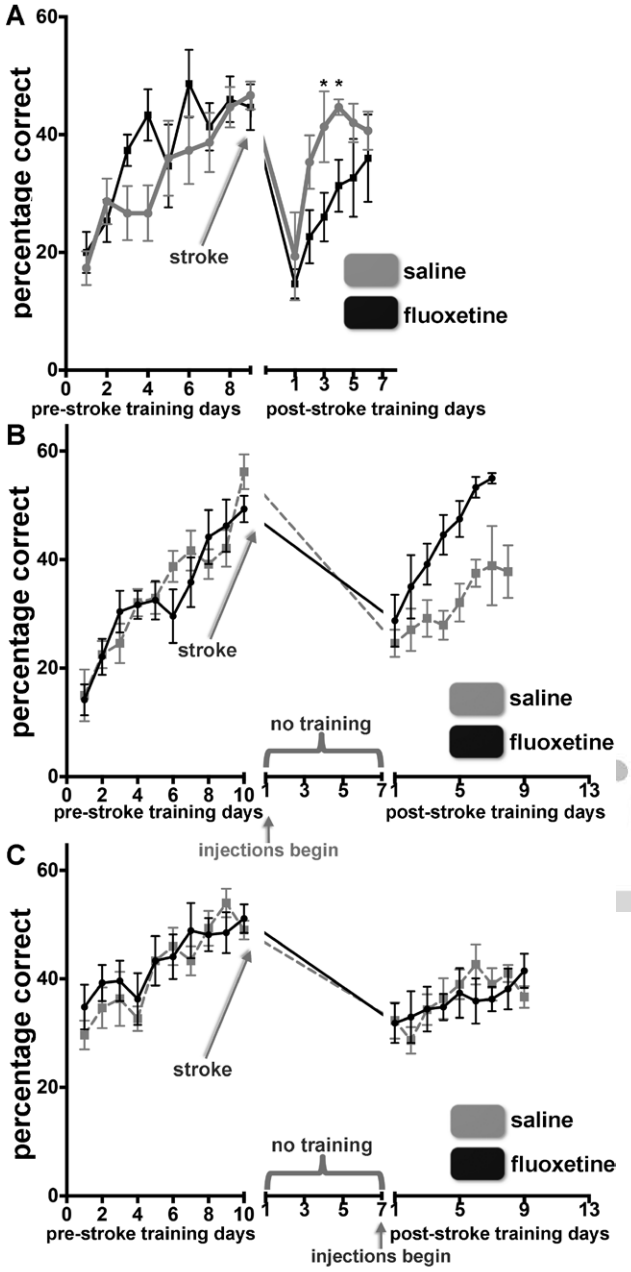

Figure 2. Fluoxetine administration beginning 24 hours after caudal forelimb area (CFA) stroke extended the sensitive period for recovery. Plots show reaching success (mean±SEM). **A**, Skilled prehension recovered completely if daily fluoxetine injections were begun 24 hours after stroke and training was begun after 1-day (n=5). Saline injected mice (re-plotted from Figure 1A) shown for comparison. **B**, Skilled prehension recovered completely even after a 7-day training delay if the mice received daily fluoxetine (black, n=9) beginning 24 hours after stroke. This was not seen in the saline group (gray, n=8). **C**, Skilled prehension did not recover after a 7-day training delay if the fluoxetine (black, n=9) was started at 7 days after stroke, ie, no different from the saline group (gray, n=10). Statistics reported in Results.

Fluoxetine Administration After Stroke Did Not Reduce Stroke Volume or Neuronal Death

Fluoxetine has been shown to be neuroprotective if given within hours after ischemic stroke.[33–35] To determine whether fluoxetine administration had an effect on neuronal death or infarct volume, which could have confounded our results, we performed 2 controls. First, to assess neuronal survival within the stroke core, we used Fluoro-Jade C staining, which makes use of a cationic fluorescent dye empirically demonstrated to bind to degenerating neuron cell bodies, dendrites, and axons after tissue fixation and provides a reliable and quantifiable index for assessing neuronal damage.[36,37] We assessed for neuronal death at 7 days after stroke. We chose this time point because (1) it would reflect a pure effect of the drug before the

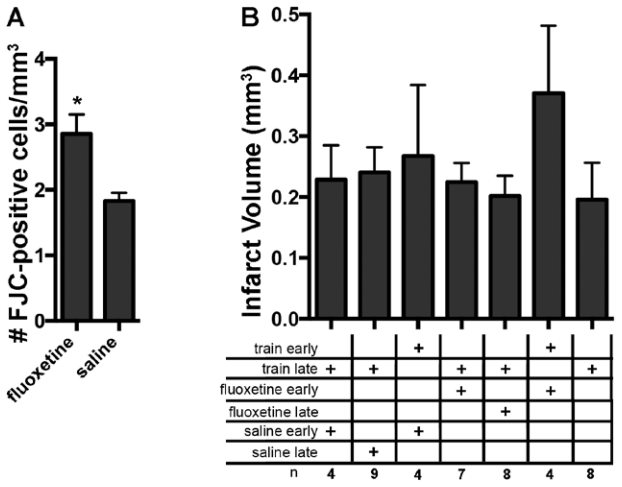

Figure 3. Fluoxetine did not reduce cell degeneration or infarct volume. **A**, Mice that received fluoxetine beginning 1-day after caudal forelimb area (CFA) stroke had increased neuronal degradation compared to mice receiving saline, indicated by Fluoro-Jade C quantification (n=6 for both conditions; *P=0.0043). **B**, There was no significant difference in CFA infarct volumes across all groups. Number (n) of mice used for volumetric analyses shown.

onset of training and (2) Fluoro-Jade C staining remains positive for at least 7 days. Quantification showed that there were more Fluoro-jade C puncta in mice receiving daily fluoxetine administration beginning 24 hours after stroke when compared with mice receiving daily saline (Figure 3A).

As a second control, mice were euthanized within 2 hours of their last training session, and stroke volumes were measured. Regardless of timing, there were no significant stroke volume differences between animals receiving no drug (ie, the animals graphed in Figure 1B), animals receiving daily fluoxetine, or animals receiving daily saline (Figure 3B). Combined, these data indicate that the beneficial effect of fluoxetine on poststroke recovery mechanisms is not attributable to either a neuroprotective effect, the first week after stroke (in fact, there was more neuronal death in the fluoxetine group), or a stroke volume reduction.

Fluoxetine Administration After Stroke Decreased Inhibitory Marker Expression in a Medial Premotor Area

We have previously demonstrated that medial premotor cortex (AGm) mediates recovery after a stroke in motor cortex, and that this is associated with decreases in parvalbumin, calbindin, and calretinin expression in this area. This suggests that modulation of region-specific excitatory/inhibitory balance is important for recovery.[25] To test whether the observed effects of fluoxetine occur through a similar mechanism, we performed blinded quantification of cells expressing parvalbumin, calbindin, and calretinin in AGm 7 days after CFA stroke. There was a statistically significant decrease in ipsilesional AGm parvalbumin expression (as well as a trend toward decreased calbindin expression) in animals that received daily fluoxetine beginning 24 hours after stroke when compared with contralesional AGm and compared with animals receiving daily saline injection beginning 24 hours after stroke (Figure 4). Alternatively, however, an increase in contralesional AGm parvalbumin expression could have just given the impression of decreased ipsilesional AGm parvalbumin expression. To rule out this possibility, we performed a comparison with historical controls in which parvalbumin expression was measured in mice who underwent sham stroke and subsequent prehension training.[25] There was a significant decrease in ipsilesional AGm parvalbumin expression in animals with stroke (23.27±0.81 mm^3) versus sham stroke (31.56±3.7 mm^3), which indicates that parvalbumin expression ispsilesional to stroke in animals treated with fluoxetine was indeed lower than baseline. A potential objection, however, is that the animals in the sham group underwent poststroke prehension training, whereas the animals in the stroke plus fluoxetine groups did not, raising the possibility that training alone may lead to a generalized increase in parvalbumin expression. However, we find this highly unlikely because (1) previous studies have shown decreases and not increases in parvalbumin expression after learning in normal animals. A training-induced decrease in parvalbumin expression in AGm would make our claim even stronger because fluoxetine would have needed to decrease this expression even further. (2) Other studies have demonstrated a similar number of parvalbumin-positive neurons in normal motor cortex as we observed in the sham stroke group.[38] Fluoro-Jade C staining of AGm revealed no immunofluorescence, which indicates that there was no death of parvalbumin interneurons (data not shown).

Previous work has shown that 3 weeks of fluoxetine administration leads to decreased parvalbumin expression in the mFC.[29] However, 7 days of fluoxetine or saline administration after stroke revealed no change in the number of cells expressing parvalbumin in the mFC either ipsilesional or contralesional to CFA stroke (data not shown). These data indicate that poststroke fluoxetine administration alters parvalbumin expression in the ipsilesional premotor area, and that these changes are anatomically specific and unrelated to training.

Discussion

Using a mouse stroke model, we first showed that there is a gradient of diminishing responsiveness to training within the first week after CFA stroke. We then showed that daily fluoxetine administration poststroke mitigated this gradient and was associated with full recovery of prehension accuracy even if training was delayed by a week. Fluoxetine was associated with decreased medial premotor inhibitory interneuron expression but did not reduce either stroke volume or neuronal death.

Poststroke Sensitive Period

Here, we demonstrated the existence of a poststroke sensitive period in mice although our data do not indicate when this sensitive period ends because motor training after 7 days still led to a small increase in poststroke motor performance that then reached plateau despite 19 days of training. This lack of responsiveness to further training is consistent with studies in the rat that show no benefit of later tune-ups, despite an initial response to training earlier after stroke.[39] That the impact of training falls off rapidly within 1 week post stroke is also consistent with results in rats showing only a modest response to training and enrichment begun after 2 weeks post stroke, compared with when begun early.[5]

It is possible that increasing the dose of training per day could have led to further gains even with a delay of 7 days. The idea that there is a dosage threshold for any given level of plasticity, below which there will be no behavioral response, is supported by the finding in rats that when training was begun 5 days post stroke, 140 reaches per day led to no improvement but 240 reaches did.[40] The existence of such a dose by time interaction is entirely consistent with the existence of a window within which there is a gradient of diminishing responsiveness to a fixed amount of training.

Human data also suggest a short-lived plasticity window after stroke, with most spontaneous recovery occurring in the first 3 months.[2,41] More recently, we have shown that the degree of recovery from impairment at 3 months follows a predictable proportionality rule: most patients, except a subset of patients with severe hemiparesis, regain ≈70% of their maximal potential recovery from their initial impairment level.[1,42]

There is an increasing evidence that there are qualitative and quantitative differences in the cortical milieu

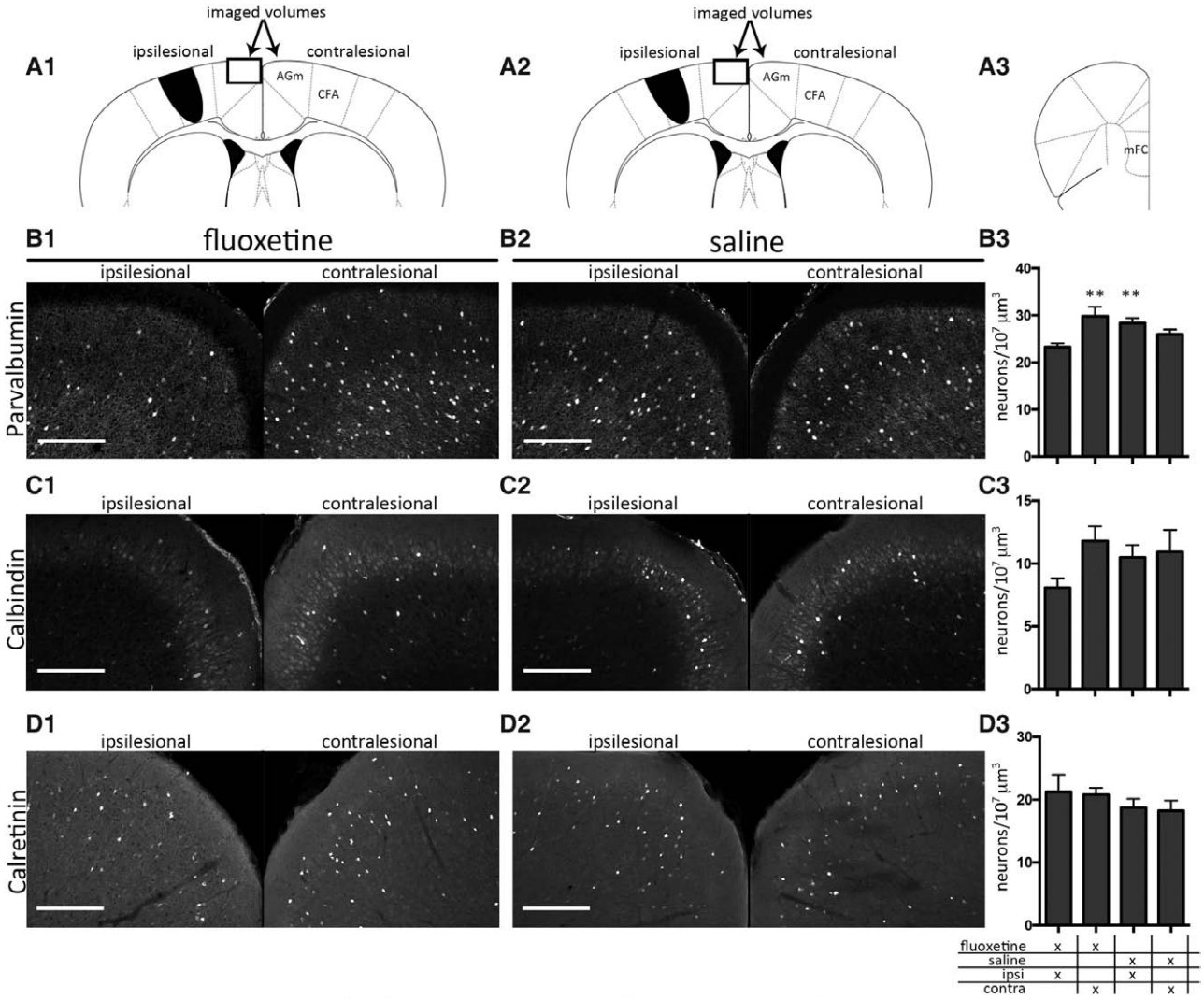

Figure 4. Fluoxetine after stroke was associated with decreased inhibitory interneuron marker expression in medial premotor cortex (AGm). **A**1 and **A**2, Coronal schematics of mouse brain showing caudal forelimb area (CFA) stroke and medial premotor cortex (AGm) (box). **A**3, Schematic of medial frontal cortex (mFC), which is approximately 1.3 mm anterior of the imaged motor regions. Representative immunofluorescent images labeled with PV (**B**1, **B**2), CB (**C**1, **C**2), or CR (**D**1, **D**2) in animals receiving fluoxetine (**B**1, **C**1, **D**1), or saline (**B**2, **C**2, **D**2), both contra- and ipsilesional relative to CFA stroke (**B**3, **C**3, **D**3). Number of PV (**B**3), CB (**C**3), and CR (**D**3) positive neurons per 107 mm^3 in the AGm. Ipsi refers to cortex ipsilesional to stroke and contralateral to the reaching paw; contra refers to cortex contralesional to stroke and ipsilateral to the reaching paw. n=6 for all conditions. Scale bars, 200 µm. ***P*=0.001 compared relative to ipsilesional cortex in animals receiving fluoxetine; no other comparison was statistically significant.

when training occurs in the poststroke sensitive period when compared with similar training in healthy subjects or in chronic stroke.[9] For example, multiple studies of the cortical environment early after stroke have demonstrated evidence for a shift in the cortical excitatory–inhibitory balance,[43] either through an increase in excitability[44–46] or a decrease in inhibition.[25,45,47] Moreover, direct manipulation of excitatory–inhibitory balance in rodents promotes axonal and dendritic growth and enhances recovery after stroke.[11,48] These data suggest that an initial reduction in inhibitory tone (especially phasic inhibition) enhances plasticity but then normalizes, which limits further large-scale cortical reorganization in response to sensory input or training.[49,50] Time-dependent changes in both glial scar and in molecules that regulate regrowth of axons through damaged tissue are also likely to be important.[51,52]

Interaction Between Fluoxetine and the Poststroke Sensitive Period

Here, we found that fluoxetine given 24 hours after stroke rescued poststroke mice from the only minimal gains expected when training is delayed by 7 days. As in a previous report, this rescue did not occur if fluoxetine was only started at 7 days,[53] which suggests that its effect is itself dependent on conditions in the sensitive period. Although earlier work has suggested a neuroprotective effect for SSRIs, especially within hours of an ischemic insult,[33,34,54,55] we found no evidence for either reduced infarct volume or reduced cell death.

Early after stroke, mice receiving daily fluoxetine performed worse when compared with mice receiving saline (Figure 2A). This is similar to previous studies showing acute fluoxetine administration leads to poorer performance on motor and spatial learning tests[56] and could be related to effects of fluoxetine

on nonhippocampal-dependent learning mechanisms.[57] It is also possible that the increased cell death in mice receiving fluoxetine (Figure 3A) played a role in the initial poorer performance. Although most studies have indicated that SSRIs tend to potentiate cell survival, a few have demonstrated fluoxetine-induced growth factor reduction[58] and SSRI-induced apoptosis, especially of diseased cells,[59–62] perhaps by potentiation of the immune system.[63] Nevertheless, despite increased cell death, animals receiving fluoxetine eventually outperformed animals receiving saline. This is similar to previous reports in which rehabilitation-induced increases in stroke volume were associated with improved behavioral outcomes,[64,65] reflecting a possible pruning effect, whereby energy-compromised neurons are eliminated early on as a result of use-dependent activation associated with rehabilitation.[6]

There is accumulating evidence that SSRIs enhance central nervous system plasticity mechanisms.[66] An intriguing mechanism that could link fluoxetine and stroke recovery is SSRI-induced disinhibition.[22,23,29,67,68] In previous work, we have shown a correlation between training-induced recovery from CFA stroke and a reduction of parvalbumin expression in a medial motor area.[25] We conclude that decreased parvalbumin expression indicates a reduction in inhibition based on the following: (1) The changes were region specific, occurring only in relevant motor areas and not in mFC (which is a nonmotor association cortex), (2) The changes occurred in the absence of cell death, and (3) parvalbumin immunoreactivity marks the activity of an inhibitory interneuron.[69,70] This conclusion, however, remains conjecture in the absence of direct physiological measurements.

Fluoxetine administration led to a decrease in poststroke parvalbumin expression in the ipsilesional medial premotor cortex in the absence of training, but the behavioral benefit only manifested with subsequent training. Previous studies suggest that pharmacological interventions must be paired with training to induce behavioral changes.[18,71–74] For example, fluoxetine enhances plasticity in fear circuits when combined with environmental experience, reshaping behavioral responses.[75] The influence of fluoxetine on inhibition may operate directly or indirectly, for example, via brain derived neurotrophic factor.[76,77]

It is unlikely that the effect of fluoxetine reported here is attributable to an effect on mood because this would predict a benefit regardless of when it was administered, which was not what was observed. Furthermore, our data are consistent with previous reports showing that fluoxetine administration for 7 days, and at dosages similar to those used in our study, is not associated with changes in mood in rodents.[78–80]

The lack of efficacy of fluoxetine when started at 7 days post stroke is not likely attributable to the effect of ischemia on the blood–brain barrier. This is because it seems that fluoxetine acts to promote reorganization in undamaged cortical areas (eg, the medial premotor area); fluoxetine has good blood–brain barrier penetration in the absence of injury[81–83] and can produce a similar decrease in parvalbumin in uninjured wild-type mice.[29] Most importantly, our main hypothesis was that the lack of responsiveness to training at 7 days can be reversed by fluoxetine given on day 1 post stroke. That this did not happen when it was given at 7 days does not detract from the principal demonstration that the temporal gradient of decreasing responsiveness to training was reversible.

Comparison to Motor Recovery in Humans

It is of interest to compare and contrast our results in mice with the Fluoxetine for Motor Recovery After Acute Ischaemic Stroke (FLAME) study in humans.[18] Patients who received fluoxetine by poststroke day 5 to 10 showed significantly greater recovery from motor impairment at 3 months than patients who received placebo.[18] A key methodological difference between our mouse study versus the FLAME study is that we pretrained our animals on the task with which their poststroke recovery was subsequently assessed, whereas in FLAME, patients were assessed with the Fugl–Meyer Scale, on which they were not pretrained. Why did the mice not spontaneously recover prehension with fluoxetine but the patients got better without training on the assessment task? There are many possibilities. First, rodents do spontaneously recover natural behaviors.[5] It could be argued that prehension is overlearned in humans, whereas it is a difficult and novel behavior for rodents. Second, the Fugl–Meyer scale can be contaminated by strength improvements.[84] Third, patients in FLAME received rehabilitative therapy, which might overlap with components of the Fugl–Meyer Scale. It is of further interest that if one calculates the expected proportional recovery[1] of 70% of maximal potential Fugl–Meyer in the fluoxetine and placebo groups in the FLAME study, the fluoxetine group on average showed full proportional recovery, which suggests fluoxetine nudged them into full spontaneous recovery.

Equating the sensitive period in rodents and humans is difficult because of size, metabolism, and central nervous system differences. Nevertheless, comparison of human[1,2,41] and rodent data[5] suggests that the rodent sensitive period closes at least 3× faster than that in humans, which is good news because it suggests that there is more time available to intervene in patients. Overall, existing data provide compelling evidence for a poststroke sensitive period in both humans and in rodent models. Future electrophysiological studies will be required to demonstrate decreased inhibition in premotor areas after a stroke in primary motor cortex. Prolonging the postsensitive period pharmacologically, as was done here in mice, holds great promise for human neurorehabilitation.

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Disclosures

None.

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Fluoxetine Maintains a State of Heightened Responsiveness to Motor Training Early After Stroke in a Mouse Model

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