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Neuronal Stem Cell and Drug Interactions: A Systematic Review and Meta-Analysis: Concise Review

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ABSTRACT

Stem cell therapy is a promising treatment option for neurodegenerative diseases that mostly affect geriatric patients who often suffer from comorbidities requiring multiple medications. However, not much is known about the interactions between stem cells and drugs. Here, we focus on the potential interactions between drugs used to treat the comorbidities or sequelae of neurodegenerative diseases and neuronal stem cells to reveal potential effects on drug safety and efficacy. To determine the potential effects of drugs frequently used in geriatric patients (analgesic, antibiotic, antidepressant, antidiabetic, antihyperlipidemic, and antihypertensive drugs) on neuronal stem cell differentiation and proliferation, we systematically searched PubMed to identify nonreview articles published in English in peer-reviewed journals between January 1, 1991, and June 7, 2018. We identified 5,954 publications, of which 214 were included. Only 62 publications provided the complete data sets required for meta-analysis. We found that antidepressants stimulated neuronal stem cell proliferation but not differentiation under physiologic conditions and increased the proliferation of stem cells in the context of stress. Several other potential interactions were identified, but the limited number of available data sets precludes robust conclusions. Although available data were in most cases insufficient to perform robust meta-analysis, a clear interaction between antidepressants and neuronal stem cells was identified. We reveal other potential interactions requiring further experimental investigation. We recommend that future research addresses such interactions and investigates the best combination of pharmacological interventions and neuronal stem cell treatments for more efficient and safer patient care. STEM CELLS TRANSLATIONAL MEDICINE *2019;8:1202–1211*

SIGNIFICANCE STATEMENT

Since drugs frequently used in geriatric patients can influence the behavior of neuronal stem cells, which are a promising therapeutic option for the treatment of neurodegenerative diseases, this study aimed to identify the potential interactions between neuronal stem cells and drugs described in the literature. Although surprisingly few studies reported data on such effects, meta-analysis revealed a clear interaction between antidepressants and the proliferation capacity of neuronal stem cells. Therefore, both future cell therapeutic approaches and pharmacological interventions need to be coordinated thoroughly to create more efficient, safer, and ultimately successful therapeutic strategies.

INTRODUCTION

Aging is the main risk factor for neurodegenerative diseases [1]. More than 20% of adults at the age of 60+ years suffer from mental or neurological disorders. This number is expected to double in individuals of over 70 years [2, 3]. In addition, there has been a tremendous rise in the number of geriatric patients suffering from mental or neurological disorders during the last decade, which is even expected to increase as our population ages [4]. Unfortunately, conventional pharmaceutical interventions for neurodegenerative diseases are often limited in efficacy [5–9]. This has encouraged the search for alternative therapeutic approaches, with neuronal stem cell therapies being among the most promising options [10]. Although clinical translation has not yet been achieved, numerous preclinical studies using neuronal stem cells provided encouraging results [10–13].

Geriatric patients are the primary patient population to benefit from prospective stem cell-based approaches to counter neurodegenerative

diseases. As older people often suffer from several chronic diseases, including hypertension, diabetes, chronic pain, and depression [14], it is relevant to consider the prevalence of polypharmacy in the target patient population [15]. The primary challenge of the inevitable combination of neuronal stem cells and drugs in clinical practice is to yield beneficial, potentially synergistic effects while avoiding detrimental ones. Therefore, a deeper understanding of the functional mechanisms of each drug and their interactions with neuronal stem cells is an important prerequisite for successful combination therapies [16]. Although this aspect has not been systematically investigated for neuronal stem cells, research in the cardiac field indicates the existence of such interactions and their considerable complexity [17].

In this study, we hypothesized that there are interactions between neuronal stem cells and drugs frequently used in geriatric patients. We intentionally choose the term "neuronal stem cells" to distinguish it from "neural stem cells," which can differentiate into neuron and glia, since neurons are the primary focus of stem cell therapy in the brain. We performed a systematic review to identify: (a) the effects of drugs on neuronal stem cell proliferation and differentiation; (b) potential differences in exerting those interactions according to drug classes, subclasses, or particular drugs; and (c) the mechanisms underlying drug–stem cell interactions.

Methods

We conducted a systematic review according to the guidelines for Preferred Reporting Items for Systematic Reviews and Meta-Analyses [18].

Search Strategy and Selection Criteria

We searched for publications listed in PubMed describing the effect of drugs frequently used in geriatric patients on neuronal stem cells. A detailed search query is provided in the Supporting Information. Publications made between January 1, 1991, and June 7, 2018 were included. We chose the start date based on when stem cells started to become widely explored as potential therapeutics. Data from pathological cells (e.g., tumor cell lines) and nonmammalian species were excluded. We included *in vitro* and *in vivo* studies as well as clinical trials of the peripheral and central nervous system (including the retina). Only publications in peer-reviewed journals containing primary data were used for analysis. Review articles, articles without full text accessibility, and non-English articles were excluded.

Selection of Publications and Data Extraction

One author (M.I.) screened the abstracts and all authors subsequently reviewed the full-text versions of the potentially eligible publications. In case of doubt, publications were discussed in consensus meetings with two other authors (M.Z. and J.B.). After screening, a quality synthesis was performed. It included all aspects referring to the internal validity of the publications such as the reporting of outliers, technical or biological replicates, and blind assessment of outcome. The distribution of drugs, samples, and the effect of the drugs on the outcome parameters were determined. Where data were stated in the text, numerical values were extracted. When a study reported several experiments, each experiment was considered as an independent experiment. Only the concentration of the drug exerting the largest effect on the stem cells and the final time point of the experiment were included in the data set.

We discriminated three distinct conditions under which the data were gathered: (a) "physiologic," in which the physiological state of neuronal stem cells was investigated, without any modification of the cells or animals during the experiment; (b) "injury" (including mental disorders), where the sample (i) mimicked a phenotype of disease (as disease models) or (ii) received a psychological challenge such as depression or a harmful or negative physical stimulus (e.g., pain); and (c) "modified," in which the animals were either genetically modified (transgenic), were housed in an enriched environment, or exposed to a combination of drugs. We identified proliferation by bromodeoxyuridine, Ki67, 3H-thymidine, 5-iodo-2-deoxyuridine staining and differentiation by detection of doublecortin, neuronal nuclei, neuron-specific class III β-tubulin, ionized calcium-binding adaptor molecule 1, nestin, glial fibrillary acidic protein, microtubule-associated protein 2, or β-III tubulin.

For meta-analysis, two authors (M.I. and A.P.) independently extracted the relevant data from the included publications. We collected data on sample size, mean, standard deviation (SD), *p*-value, statistical analysis, and the reported mechanism underlying the action of the drugs on neuronal stem cells. We contacted the authors of the publications that did not provide the complete data set to collect the missing information. In case the data were only available as graphs, we performed graphical measurement using ImageJ (version 1.51S, RRID:SCR_003070) as previously described to calculate the means and SDs [19].

Statistical Analysis

To compare data from the different publications, we used the standardized mean difference (SMD) since the measurement units of proliferation and differentiation were very diverse among the publications. Hedge's *g* SMD with correction factor was chosen due to the small sample size (below 20 samples for each study). We applied the partitioning of heterogeneity to determine the significance of the reported study quality explaining differences in observed efficacy. We calculated an estimate of the effect size based on the visual assessment of the forest plot and I^2 value by the DerSimonian and Laird random effect model meta-analysis. A confidence interval (CI) of 95% was applied. We generated the analyses using Cochrane's Review Manager Software for meta-analysis (*RevMan* version 5.3, RRID:SCR_003581) as well as manually in Excel as previously reported [20]. An exemplary calculation can be found in Supporting Information and the complete Excel calculation sheet in the Supporting Information xls. A probability value of $p < .05$ was considered statistically significant, except for the subgroup analysis where the obtained *p*-values were compared with the Holm–Bonferroni cutoff *p*-value to correct for multiplicity [21]. The Holm–Bonferroni cutoff *p*-value is calculated as follows: (target α [=0.05])/(k − rank number of pair [by degree of significance] + 1), where k is the number of tests.

Results

After the screening of 5,954 publications, we identified 214 eligible publications, of which 115 were records in the physiologic,

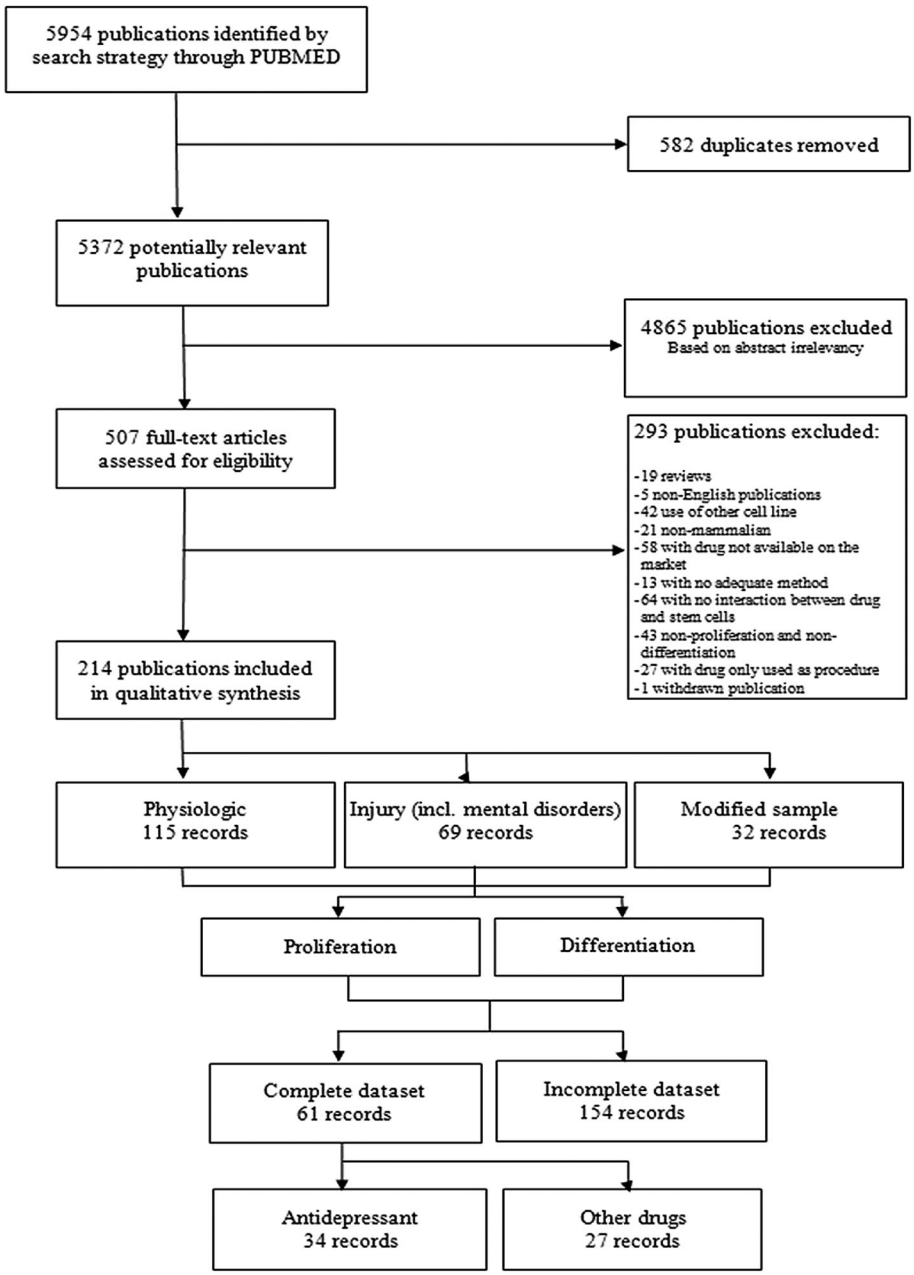

Figure 1. Flow diagram of the systematic search according to the guidelines for Preferred Reporting Items for Systematic Reviews and Meta-Analyses [18]. Of note, the number of "records" does not equal the number of publications due to the fact that some experimental designs included multiple experiments, such as physiologic versus injury or physiologic versus modified conditions, representing different "records".

69 records in the injury, and 32 records in the modified condition (Fig. 1, Supporting Information Table S1). The distribution of drug classes, subclasses, and individual drugs among all conditions produced some predominant clusters especially for antidepressants and analgesics (83 and 40 number of records, respectively; Tables 1 and 2). The records in the injury (including mental disorders) and modified conditions were very heterogeneous (Supporting Information Table S2). Among all conditions, we found that more than two-thirds of the publications (148 of 214 publications, 69.2%) used hippocampal stem cells, but no record reported that neuronal stem cells were transplanted into an animal model or patient while assessing the effect of drugs used in geriatric patients on neuronal stem cells (Supporting Information Table S3).

Drug Effects on Neuronal Stem Cells

Table 3 shows the number of publications reporting stimulating, neutral, and inhibiting effects on the proliferation and differentiation of neuronal stem cells for each drug class summarizing all conditions. Supporting Information Table S4 presents equivalent information only under physiologic conditions. Antidepressants had a predominantly stimulating effect on neuronal stem cell proliferation and differentiation while analgesics showed the opposite effect in all conditions. Similar findings were obtained

Table 1. Distribution of the records of drug classes and subclasses

Drug class	Drug subclass	Number of records
Analgesic	Opioid	25
	Cyclooxygenase-2 inhibitor	8
	Nonsteroidal anti-inflammatory drug	7
	Total	**40**
Antibiotic	Aminoglycoside	9
	Macrolide	9
	Quinolone	6
	Tetracycline	4
	Cephalosporin	2
	Nitroimidazol	1
	Total	**31**
Antidepressant	Selective serotonin reuptake inhibitor	54
	Tricyclic antidepressant	22
	Monoamine oxidases inhibitor	5
	Atypical antidepressant	1
	Selective serotonin-norephinephrine reuptake inhibitor	1
	Total	**83**
Antidiabetic	Insulin	9
	Thiazolidinedione	9
	Incretin mimetic	3
	Nonsulfonylurea	1
	Total	**22**
Antihyperlipidemic	Statin	6
	Total	**6**
Antihypertensive	Loop diuretic	4
	Aldosterone receptor inhibitor	3
	Alpha 2 adrenergic agonist	3
	Beta blocker	3
	Calcium channel antagonist	3
	Ace inhibitor	2
	Angiotensin II receptor inhibitor	1
	Total	**19**
Other drugs	Phosphodiesterase type-5	6
	Corticosteroid	4
	Hormonal therapy	2
	Rho-kinase inhibitor	2
	Supplement	2
	Antihelminthic	1
	Atypical antipsychotic	1
	Cytosine arabinoside	1
	Triazole derivative	1
	Total	**20**

when looking at the physiologic condition alone. For the other drug classes, no predominant effect was observed (Table 3, Supporting Information Table S4).

Table 2. The six most frequently used drugs identified by the systematic search

Drug class	Drug subclass	Drug	Number of records
Antidepressant	Selective serotonin reuptake inhibitor	Fluoxetine	44
Analgesic	Opioid	Morphine	19
Antidepressant	Atypical antidepressant	Imipramine	18
Antidiabetic	Insulin	Insulin	12
Antibiotic	Macrolide	Rapamycin	8
Antidiabetic	Thiazolidinedione	Rosiglitazone	6

We further divided the drug classes into different subclasses and individual drugs to identify differences within a drug class. However, neither specific drugs nor subclasses mediate different effects compared with the main drug classes (compare Table 3 with Supporting Information Table S5).

Meta-Analysis

Statistical data such as sample size, mean, and SD are required to perform meta-analysis. Overall, we identified 61 data sets reporting complete information. First, we extracted 42 complete data sets from the publications. Second, we obtained 19 additional data sets after contacting the authors of the publications that do not contain all of the aforementioned data (we only contacted the authors when five or more records were available per condition and drug class, our predefined threshold to perform meta-analysis). Third, we measured the mean and SD directly from the respective graphs of 24 additional publications. Those only stated the sample size and their authors did not respond to inquiries. With all other data sets, at least one parameter was missing to calculate the effect size.

Only the data of the antidepressant drug class were sufficient for meta-analysis, of which 21 records described the effect on proliferation and seven on differentiation in the physiologic condition, whereas six records were on proliferation in the depression condition (Supporting Information Tables S6–S8). Meta-analysis confirmed that antidepressants significantly stimulated neuronal stem cell proliferation in the physiologic condition (Hedges' g SMD, 0.66; 95% CI, 0.20 to 1.12; p = .005, Fig. 2A). The most frequently studied antidepressant subclass, selective serotonin reuptake inhibitors (Table 2), also significantly induced proliferation of neuronal stem cells (Hedges' g SMD, 0.72; 95% CI, 0.17–1.27; p = .01 < .017 [Holm–Bonferroni cutoff p-value], Fig. 2A). We also performed meta-analysis on the effect of antidepressants on neuronal stem cell differentiation, which was not significantly changed (Hedges' g SMD, 0.23; 95% CI, −0.68 to 1.13; p = .63, Fig. 2B). Furthermore, there was no statistically significant evidence that antidepressants stimulate stem cell proliferation in models of depression (Hedges' g SMD, 1.14; 95% CI, −0.03 to 2.32; p = .06, Fig. 3).

Potential Effect of Drugs on Neuronal Stem Cells in the Context of Brain Injury

Some publications offer insights into the potential effect of drugs on neuronal stem cells in the context of brain injury that may be informative for future research. We found 20 records

Table 3. Distribution of the drug classes based on the effect on neuronal stem cells

	Proliferation			Differentiation		
Drug classes	**Stimulating**	**Neutral**	**Inhibiting**	**Stimulating**	**Neutral**	**Inhibiting**
Analgesic	6 (19.3%)	5 (16.1%)	20 (64.5%)	6 (28.6%)	2 (9.5%)	13 (61.9%)
Antibiotic	8 (34.8%)	5 (21.7%)	10 (43.5%)	6 (24.0%)	7 (28.0%)	12 (48.0%)
Antidepressant	39 (65.0%)	15 (25.0%)	6 (10.0%)	30 (56.6%)	13 (24.5%)	10 (18.9%)
Antidiabetic	3 (37.5%)	3 (37.5%)	2 (25.0%)	9 (47.4%)	4 (21.0%)	6 (31.6%)
Antihypertensive	7 (58.3%)	3 (25.0%)	2 (16.7%)	7 (63.6%)	2 (18.2%)	2 (18.2%)

The number of publications reporting a stimulating, inhibiting or neutral effect on neuronal stem cell proliferation or differentiation is given. Relative percentages per drug class are indicated in brackets.

investigating drug–stem cell interactions in *in vivo* and *in vitro* models of brain ischemia and hypoxia. For instance, the phosphodiesterase type-5 inhibitor sildenafil stimulated proliferation of neuronal stem cells (five records). We cannot exclude that the injury condition itself influences drug–stem cell interactions, but in the case of sildenafil, the stimulating effect on neuronal stem cell proliferation was also found under physiologic conditions. However, the overall number of publications with complete data sets and the heterogeneous effects were too low to perform robust meta-analysis in the brain injury subgroup.

Discussion

Our systematic review revealed that the effects of drugs used in geriatric patients on neuronal stem cells have not been studied in much detail so far. In fact, the identified publications reported such interactions as an auxiliary finding. Relatively few publications exist on a limited number of drugs, and their heterogeneity was high with respect to the type of experiment (*in vivo* or *in vitro*), condition under which the drugs were assessed (physiologic, injury, or modified) and the investigated drugs (Tables 1, 2, Supporting Information Tables S2, S3). We intentionally chose to investigate neuronal stem cells in their various types and applications because we wanted to provide a comprehensive overview about the interactions of neuronal stem cells and drugs *in vitro*, *in vivo*, and in clinical trials. We found that, although there are numerous studies using *in vitro* and *in vivo* models, there is no clinical trial investigating drug–stem cell interactions. In addition, we only found studies in cultured neuronal stem cells or endogenous stem cell populations *in vivo* (Supporting Information Table S3). In those studies that investigated transplanted cells, only mesenchymal stem cells, but not neuronal stem cells were used [22].

Nevertheless, we were able to show a clear interaction between antidepressants and neuronal stem cells under physiologic conditions and in models of depression (Figs. 2, 3). The results obtained by studies using well-suited animal models may be relevant for clinical treatment. Antidepressants may serve as an example: in case their class effects on proliferation and differentiation of neuronal stem cells was proven for particular antidepressants, those may be considered as the treatment of choice for poststroke depression even in case alternative drugs may provide better primary antidepressant effects, but less regenerative stimuli. However, the situation may be far more complex in human patients. It is important to understand that proliferation and differentiation were chosen as the preset criteria for stem cell function in our analysis. Although important for stem cell function, these parameters are neither the only ones indicating improved functional recovery after stroke, nor the most important ones. This is underlined by the recently published, neutral results of the Fluoxetine or Control Under Supervision trial study [23]. Although fluoxetine was effective in preventing poststroke depression, there were no obvious effects of functional recovery, but a higher rate of bone fractures as an adverse event [24].

Further investigations regarding the modes of action of the drugs revealed functional hypotheses for pathways underlying their effects on neuronal stem cell differentiation and proliferation (Fig. 4). Verifying those and elucidating the underlying mechanisms is an important step to develop more effective and specific drug–stem cell combination treatments and to minimize potential adverse effects.

Potential Mechanisms Affecting Proliferation and Differentiation

In order to understand the drug effects on neuronal stem cells, we also assessed the underlying mechanisms investigated in the included publications. Among all records in the physiologic condition, the six most frequently used drugs (fluoxetine, imipramine, morphine, rosiglitazone, rapamycin, and insulin, Table 2) have been tested for their mechanism of action. However, the identified pathways were only described in a single publication each (Fig. 4) and therefore still need to be verified:

Fluoxetine, imipramine, and morphine affect the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway [25–27]. This is one of the key signaling pathways modulating neuronal stem cell proliferation and differentiation [28]. MAPK signaling contributes to synaptic plasticity and long-term memory formation [29]. It is also supposed to be neuroprotective [30].

The antidepressant fluoxetine increased the proliferation of neuronal stem cells. This is likely mediated by the activation of serotonin-1-agonist receptor (SHT1Ar, Fig. 4) [31, 32]. SHT1Ar activates phosphatidylinositol-4,5-biphosphate 3-kinase (PI3K), followed by an increase of Akt1 that in turn increases neuronal stem cell proliferation [33]. Moreover, SHT1Ar triggers the MAPK/ERK cascade, which increases neurogenesis by stimulating cyclin D1 [31]. Hui et al. reported that SHT1Ar induces ser9, which inhibits glycogen synthase kinase 3β (GSK3β) followed by the activation of β-catenin [32]. Another potential mechanism is that SHT1Ar stimulates the cAMP response element-binding protein by activating MAPK/ERK [25]. In a study unrelated to SHT1Ar, fluoxetine stimulated cyclin-dependent kinase inhibitor protein 1 (P21/CIP1) leading to increased neurogenesis [34].

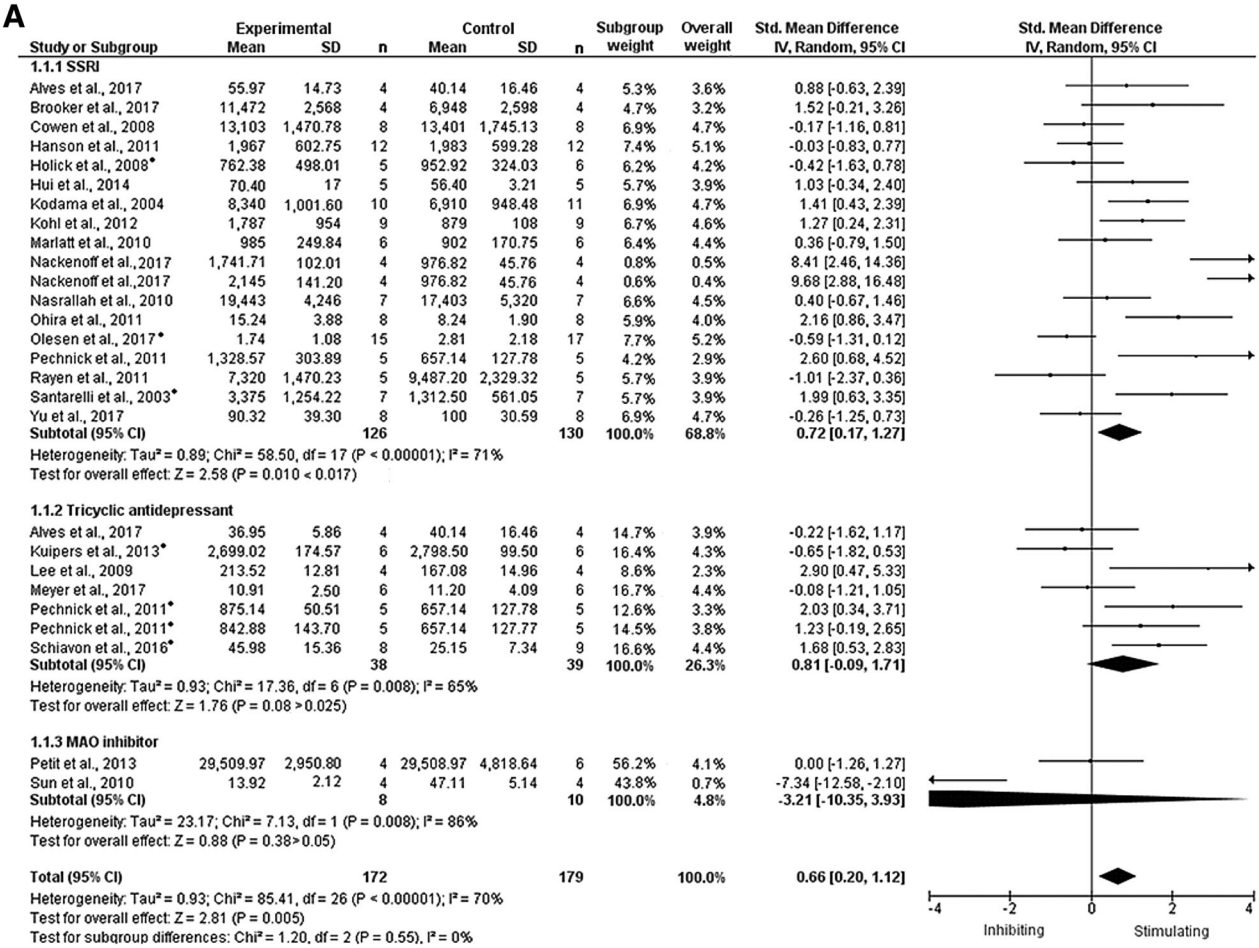

Figure 2. Forest plot of the effect of antidepressants under physiologic conditions. We found that antidepressants stimulated neuronal stem cell proliferation (**[A]** Hedges' *g* standardized mean difference [SMD], 0.66; 95% CI, 0.20–1.12; $p = .005$) but not differentiation (**[B]** Hedges' *g* SMD, 0.23; 95% CI, −0.68 to 1.13; $p = .63$) under physiologic conditions. In (A), the weights are given for both subgroup and overall analysis. The obtained *p*-values in the subgroup analysis were compared with the cutoff *p*-value calculated by the Holm–Bonferroni method that is a sequential method of testing *p*-values (from smallest to largest) to correct for multiplicity. * indicates publications from which SDs and means were derived by manual graphical measurement using ImageJ.

Rapamycin and insulin affect the mammalian target of rapamycin (mTOR) signaling pathway in different ways. Insulin stimulates mTOR and rapamycin inhibits it [35, 36]. mTOR is a receptor tyrosine kinase that is pivotal in regulating cell proliferation and differentiation [37]. The inhibition of mTOR blocks p70 ribosomal S6 kinase (S6K), which then leads to the inhibition of stem cell differentiation via telomerase activity reduction [36, 38]. S6K has been well known in regulating the cell cycle, growth, and survival [39].

The antidiabetic drug from the subclass of thiazolidinediones, rosiglitazone, stimulates the neurotrophic factor α1, which then upregulates the fibroblast growth factor-2 (FGF-2). FGF-2 induces neurogenesis in the hippocampus [23]. Another study demonstrated that FGF-2 needs cystatin C to induce its mitogenic

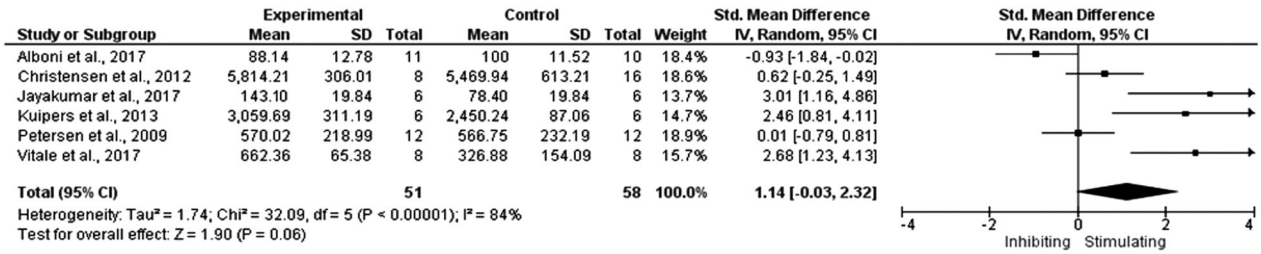

Figure 3. Forest plot of the effect of antidepressants in the models of depression. We identified that antidepressants increased the proliferation of stem cells in the context of stress; however, the effect was not statistically significant (Hedges' *g* standardized mean difference [SMD], 1.14; 95% CI, −0.03 to 2.32; p = .06). * indicates publications from which SDs and means were derived by manual graphical measurement using ImageJ.

activity [40]. Unfortunately, this was not confirmed by the identified publications.

Altogether, the pathways described to be influenced by the drugs in the identified publications fit to the results of other publications on neuronal stem cell proliferation and differentiation. However, although they are potential therapeutic targets, these pathways also control many very fundamental cell processes. Modulating these pathways may therefore cause interference with important basic cellular functions. Hence, it would be necessary to find more specific targets avoiding adverse side effects and/or supporting positive effects. In addition, prospective research should validate each pathway in the particular cell type and the source of interest.

Unmet Research Needs

A systematic screening of drugs applied in geriatric clinical routine on neuronal stem cell proliferation and differentiation is warranted. As a first step, this should be investigated under physiologic conditions to comprehend the basic interactions of drugs with neuronal stem cells. Subsequently, these mechanisms should be assessed in injury conditions, for example, animal models of neurodegenerative diseases. This is of particular relevance since a number of specialized animal models exist. This includes transgenic and immunosuppressed animals in which the brain microenvironment during degeneration or after injury can be significantly different from the wild type. Moreover, drug metabolism (pharmacokinetics and dynamics) obviously differs between mice and men. Hence, it is rationale to assume that these differences may also effect any potential interactions between drugs and neuronal stem cells. However, studies investigating drug–stem cell interactions *in vivo* are scarce, which is why we have combined all such studies in the "injury condition" category. Hence, future research should address this question systematically in relevant disease models and shall focus on the impact of animal species and strain used.

Hence, we need to ensure that the knowledge generated from animal studies is indeed translatable to the human situation. Potential approaches involve sophisticated models mimicking a human organism, such as interconnected organs-on-a-chip. Moreover, such studies should primarily focus on the combinations of stem cells with clinically applied drugs and less on purely experimental substances, and shall include comprehensive safety readout protocols.

Limitations of the Systematic Review and Meta-Analysis

Our analysis has several limitations:

i. We did not specify an *ex ante* protocol prior to the meta-analysis of the available data, including the specification of the primary outcome measure. Here, we performed meta-analyses on the effect of drugs used in the elderly and both the proliferation and differentiation of neuronal stem cells.
ii. We did not focus on drug effects on other stem cell functions such as migration and survival. The exclusion was made because migration is difficult to measure *in vivo* and it has different effects based on species differences [41]. On the other hand, survival, explicitly defined, is not a function of stem cells. On the contrary, integration is another function of stem cells and only shown in differentiated cells, therefore it was included in our study.
iii. The meta-analysis is currently quite limited due to the understudied effects of drugs on neuronal stem cells. However, despite the small sample size, our meta-analysis identified an interaction, which may indicate a strong effect, making these findings even more relevant. Nevertheless, more studies and particular analyses focusing on the therapeutically more frequently applied populations such as mesenchymal stem cells are warranted.
iv. We found only publications using neuronal stem cell cultures or investigating endogenous neuronal stem cells. Further studies investigating the effect of drugs on transplanted neuronal stem cells are necessary.
v. The heterogeneity of the samples (Table 1) limits general conclusions.
vi. Some drugs were studied more frequently than others (Table 2) which can potentially over represent a single drug from a particular class or subclass leading to result bias. For example, fluoxetine dominated among the antidepressants, accounting for more than half (53.0%) of the publications in this drug class, followed by imipramine (21.7%). However, when comparing the effect of the main drug classes with their subclasses, we did not reveal any differences (Table 3, Supporting Information Table S5). In addition, the number of publications on newer antidepressant drugs was low, for example, on sertraline (n = 1) and mirtazapine (n = 0). These drugs show better efficacy than fluoxetine [42], but may have different effects on neuronal stem cell proliferation and differentiation and should therefore be investigated as well.
vii. The overall quality of the publications was relatively poor. We rarely found information on the reporting of outliers (two publications, 0.9%). Experimental evidence for the proposed underlying mechanism was provided more frequently, but still only by one-third of all publications (41 records out of 115 records in the physiologic

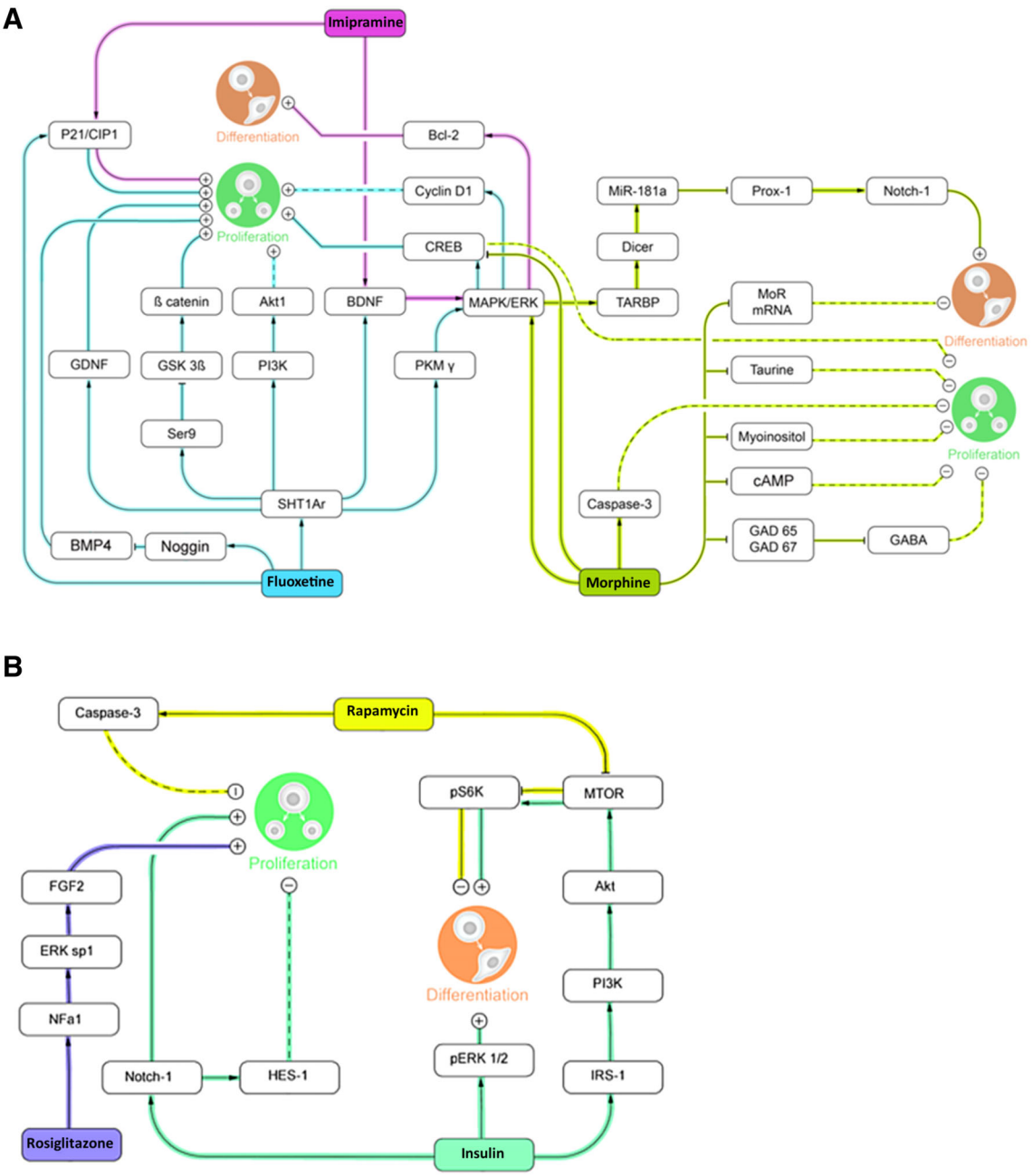

Figure 4. Recorded pathways from the selected publications. The mechanisms of the drugs **(A)** imipramine, fluoxetine, morphine, and **(B)** rosiglitazone, rapamycin, and insulin have been reported in a single publication each. Arrows indicate stimulation and T-shapes indicate inhibition of the subsequent substance. Positive signs indicate stimulation and negative signs indicate inhibition of the end effects (proliferation or differentiation). The straight lines indicate proven mechanisms and the dotted lines indicate assumed mechanisms. Abbreviations: Bcl-2, B-cell lymphoma-2; BDNF, brain-derived neurotrophic factor; BMP4, bone morphogenetic protein 4; cAMP, cyclic adenosine monophosphate; CIP1, cyclin-dependent kinase (CDK) inhibitor protein 1; CREB, cAMP response element-binding protein; FGF2, fibroblast growth factor-2; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; GDNF, glial cell-derived neurotrophic factor; GSK3β, glycogen synthase kinase 3β; HES-1, hairy and enhancer of split-1; IRS-1, insulin receptor substrate-1; MAPK, mitogen-activated protein kinase; NF-α-1, nuclear factor-α-1; pERK/ERK, phosphorylated extracellular signal-regulated kinases; PI3K, phosphatidylinositol-4,5-biphosphate 3-kinase; PKM, protein kinase M; SHT1Ar, serotonin-1-agonist receptor.

condition, 35.7%). In addition, basic statistical data such as mean and SD were sometimes difficult to extract. We have tried to minimize this weakness by contacting the authors of the respective studies to obtain mean and SD and where not possible measured them graphically.

The lack of clinical trials on drug–neuronal stem cell interactions, despite an increasing number of stem cell trials (only five trials using neuronal stem cells from a total of 120 stem cell trials in neurological disorders since January 1991, www.clinicalTrials.gov), reveals that this issue imperatively deserves more attention.

Biomarkers and imaging techniques indicating neuronal stem cell proliferation and differentiation are needed to assess these processes as secondary endpoints in clinical trials.

Conclusion

The interactions between neuronal stem cells and drugs frequently used in geriatric patients are currently understudied. Despite limited data, we were able to perform a meta-analysis for the effect of antidepressants on proliferation and revealed a clear interaction. This suggests that there may be further effects of drugs that warrant further investigation under physiologic and injury conditions. This will unravel how pharmacological interventions and neuronal stem cells can be combined in more efficient, safer, and ultimately successful therapeutic strategies.

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Author Contributions

M.I., M.Z.: conception and design, collection of data, data analysis and interpretation, manuscript writing, final approval of manuscript; A.P., D.R.: collection of data, final approval of manuscript; J.B.: conception and design, financial support, collection of data, data analysis and interpretation, manuscript writing, final approval of manuscript.

Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

Data Availability Statement

All data that support the findings of this study are available in the manuscript and supplemental data.

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