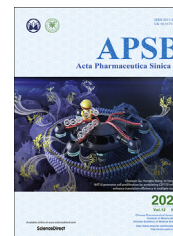




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REVIEW

# New idea to promote the clinical applications of stem cells or their extracellular vesicles in central nervous system disorders: Combining with intranasal delivery



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**Abstract** The clinical translation of stem cells and their extracellular vesicles (EVs)-based therapy for central nervous system (CNS) diseases is booming. Nevertheless, the insufficient CNS delivery and retention together with the invasiveness of current administration routes prevent stem cells or EVs from fully exerting their clinical therapeutic potential. Intranasal (IN) delivery is a possible strategy to solve problems as IN route could circumvent the brain–blood barrier non-invasively and fit repeated dosage regimens. Herein, we gave an overview of studies and clinical trials involved with IN route and discussed the possibility of employing IN delivery to solve problems in stem cells or EVs-based therapy. We reviewed relevant researches that combining stem cells or EVs-based therapy with IN administration and analyzed benefits brought by IN route. Finally, we proposed possible suggestions to facilitate the development of IN delivery of stem cells or EVs.

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## 1. Introduction

Because of the therapeutic potential in improving prognosis, stem cells and their extracellular vesicles (EVs)-based therapy is developing rapidly in the research and clinical application of central nervous system (CNS) diseases<sup>1–6</sup>. In various CNS diseases, stem cells [*e.g.*, mesenchymal stem cells (MSCs)] are found that can suppress the activation of neuroglial cells, reduce the concentration of inflammatory cytokines and reactive oxygen species (ROS) in the microenvironment, and promote the regeneration of vessels and neurons<sup>7–11</sup>. Paracrine is a vital pathway to realize the functions of stem cells. And as an important component of paracrine, EVs of stem cells are found to have similar functions<sup>12–14</sup>. Furthermore, stem cells or EVs are observed would actively accumulate in the lesion site of the CNS diseases. Hence, stem cells or EVs can also serve as carriers for drug or gene delivery in CNS disorders treatment<sup>15–18</sup>. Moreover, some progenitor cells could differentiate to replenish damaged or missing tissue in the lesion site<sup>19,20</sup>. However, the blood–brain barrier (BBB) greatly restricts entry of most cells and vesicles into the brain<sup>21–23</sup>. The complex mechanism involved in CNS disorders and the vulnerability of the CNS also increased the difficulties for treatment, the dose of stem cells or EVs is restrained and relapse of diseases is prone to happen<sup>24–28</sup>. Therefore, further improvements to stem cells or EVs-based therapy are required to realize the therapeutic potential in clinical CNS disorder management.

The administration route directly influences the pharmacodynamics and pharmacokinetics of therapeutic agents<sup>29–31</sup>. Different clinical occasions have additional demands for administration methods<sup>32,33</sup>. And as active biologics, stem cells or EVs also have more requirements for delivery compared with traditional drugs<sup>34,35</sup>. Therefore, discussion about the administration route of stem cells or EVs-based therapy in CNS disorder treatments is increasing in the recent five years<sup>36–39</sup>. The intranasal (IN) route has a unique nose-to-brain pathway and shows many advantages when delivering biologics like protein and cells<sup>34</sup>. In the past few years, studies that combined stem cells or EVs-based therapy with the IN route has increased rapidly in various fields of CNS disorder<sup>40–44</sup>. However, there is no review available to systematically introduce the up-to-date status of the combination of IN route and stem cells or EVs-based therapy and conclude benefits IN route brought to stem cells or EVs-based therapy. Herein, we analyzed problems in stem cells or EVs-based therapy in clinical trials and the necessity for a new administration approach. We gave an overview of the performances of IN delivering chemical compounds and proteins in preclinical studies and clinical trials. We then discussed the feasibility and possible transportation route of IN delivering stem cells or EVs. We reviewed researches using IN administration for stem cells or EVs delivery and concluded multi-level benefits of the combination of IN route and stem cells or EVs-based therapy. The current limitations of the IN delivery for stem cells or EVs are also analyzed. Finally, we proposed several suggestions to facilitate the development of IN delivering stem cells or EVs.

## 2. Challenges for stem cells or EVs-based therapy in CNS disorders and opportunities for intranasal route

### 2.1. Troubles in clinical translation of stem cells or EVs-based therapy

Stem cells or EVs-based therapy has become a hot spot in many research fields due to their unique features<sup>45,46</sup>. All sorts of stem

cells, like MSCs<sup>47</sup>, neural stem cells<sup>7</sup> (NSCs), pluripotent stem cells<sup>20,48</sup>, and along with EVs<sup>12,49,50</sup>, have been used in different fields like wound healing<sup>51</sup>, diabetes<sup>52</sup>, arthritis<sup>53</sup>, and especially, CNS diseases. Our previous studies found that engineered MSCs or NSCs could significantly diminish cerebral ischemia volume, reduce mortality rate, and promote motor function recovery in ischemic stroke<sup>7,8</sup>. Similarly, in a spinal cord injury (SCI) model, we observed that MSCs and EVs promoted angiogenesis and neurogenesis in the lesion site and significantly improved behavioral performance of rats<sup>12,54</sup>. In other reports, stem cells or EVs-based therapy could restore the deficit memory in Alzheimer's disease (AD) models<sup>9</sup>, rescue the loss of dopaminergic (DA) neurons in Parkinson's disease (PD) models<sup>55</sup>, normalize behavior in various psychiatric disorder models<sup>11,56,57</sup>, and inhibit progress of brain tumors<sup>58</sup>.

Encouraged by these results in animal models, clinical studies involved with stem cells or EVs also increased in recent years; some of them even proceeded into phase III of clinical trials. Table 1<sup>14,47,59,60</sup> lists recent representative clinical trials of stem cells or EVs-based therapy.

However, most stem cells or EVs-based treatments are not approved for the clinical treatment of CNS disorders<sup>61</sup>. Safety is a broad concern, yet most stem cell or EVs therapies did not show more adverse events in existing clinical and preclinical studies, even in the long-term<sup>62–65</sup>. Clinical trials of stem cells or EVs-based therapy that were withdrawn or terminated due to safety issues are not significantly higher than other studies<sup>61</sup>. The source of stem cells or EVs is regarded as another problem, since the isolation and expansion of autologous stem cells commonly used in clinical trials are inconvenient and time-consuming. However, commercial allogeneic stem cells like Stempeuce!® began available and used in clinical recently<sup>66,67</sup>. These commercial cells could be a source in the future.

Lack of efficacy, however, seems to be a big problem for stem cells or EVs-based therapy in clinical translation. Many clinical results showed stem cells or EVs treatment strategies had no or limited effect on different CNS conditions<sup>3,4,59</sup>. For examples, MSCs intravenous treatment had no or limited effect in ischemic stroke patients. Modified Rankin Scale (mRS), Barthel Index, and National Institute of Health Stroke Scale (NIHSS) showed no significantly improvement in months after MSCs administration. Although all the studies suggested application of MSCs in patients was safe<sup>59,65,68</sup>. Failures are expected to appear as stem cells or EVs-based therapy is still in its early days of clinical research. We should find the reasons for the unsatisfied efficacy and solutions for the further development of stem cells or EVs-based therapy.

Insufficient lesion site delivery and retention are possible reasons for the unsatisfied efficacy in the clinical trials of stem cells or EVs treatment<sup>61</sup>. System administration is most often used in both preclinical and clinical studies of CNS diseases. However, many studies have pointed out that less than 1% of total stem cells or EVs could reach the lesion part. Most cells are trapped in small capillaries or retained in organs like the liver and lungs<sup>69–71</sup>. The BBB would further prevent stem cells or EVs from entering the brain. Moreover, during the injection, the shear stress in syringe needles could damage stem cells or EVs<sup>72,73</sup>. The interaction of blood components like complements and lymphocytes with exogenous objects could further influence the viability of stem cells or EVs<sup>74</sup>. Therefore, stem cells or EVs that reached the lesion site may in a poor state and more vulnerable to the ROS, excitotoxicity, and activated immune cells in the lesion site's harsh microenvironment. As a result, few stem cells or EVs could retain

**Table 1** Representative clinical trials of stem cells or EVs based therapy in CNS diseases.

Condition	NCT number	Phase	Intervention	Status	Ref.
Ischemic stroke	NCT03545607	Phase III	Single intravenous infusion of multipotent adult progenitor cells	Recruiting	4
	NCT03384433	Phase I/II	Intraparenchymal injection of allogenic MSCs derived exosome	Recruiting	N/A
	NCT01716481	Phase III	Single intravenous administration of autologous MSCs	Unknown	59
Alzheimer's disease	NCT02054208	Phase I/II	Repeated intraventricular administrations of MSCs <i>via</i> an ommaya reservoir at a 4-week interval	Completed	60
	NCT04388982	Phase I/II	Repeated IN administrations of MSCs-Exos twice a week for 12 weeks	Recruiting	N/A
Parkinson's disease	NCT04506073	Phase II	Repeated administrated allogeneic MSCs every 3 months for three doses	Recruiting	N/A
Multiple sclerosis	NCT04047628	Phase III	Repeated intrathecal or intravenous administration of autologous MSCs at six-month interval for 2 doses	Completed	47
Spinal cord injury	NCT02302157	Phase I/II	Single intralesional administration of oligodendrocyte progenitor cells	Completed	N/A
	NCT04520373	Phase II	Single intrathecal administration of autologous MSCs	Recruiting	N/A
Neonatal stroke	NCT03356821	Phase I/II	Repeated IN administration of allogeneic MSCs twice within the first week of onset	Completed	N/A

Resource of the clinical trial is from [ClinicalTrials.gov](https://clinicaltrials.gov).

MSCs, mesenchymal stem cells; IN, intranasal; MSCs-Exos, mesenchymal stem cell derived exosomes; N/A, not applicable.

in the lesion site after reaching<sup>54</sup>. Research showed few days after intralesional administration, a rapid decrease in the number of stem cells happened in the lesion site<sup>54</sup>. Although even the small percentage of stem cells or EVs remained is enough to treat animal models, patients' primary physiological conditions and regenerative capacity is not as good as model animals and need a higher lesion delivery and retention. Therefore, increasing lesion site delivery efficiency and retention could help to improve the efficacy of stem cells and EV therapy in clinical.

The invasiveness and dose frequency are other possible reasons. A high-invasive administration approach may not be tolerable by CNS patients. Embolism, infection, and trauma could happen during administration<sup>75,76</sup>. Although intracerebroventricular infusion is regarded as a safe way for long-term administration, infection and other adverse events are still often reported<sup>77</sup>. Therefore, currently single-dose or doses with long-interval of stem cells or EVs are commonly used to reduce adverse events in patients, [Table 1](#) lists representative stem cells and EVs treatment regimens in clinical trials<sup>59,60</sup>. But some CNS diseases are progressive or would recurrent, frequent dosage may help to achieve long-term relief<sup>24,78</sup>. Researches showed that rescue in behavioral performance after single administration could start to diminish in days after administration in some CNS disorder models<sup>44,78,79</sup>. Therefore, repeated dosage delivered non-invasively may be a better choice for stem cell and EV therapy<sup>80,81</sup>.

In a word, 1) insufficient lesion site delivery, 2) retention and 3) patient tolerance to multi-dose regimens are several factors that influence the clinical performance of stem cells or EVs-based therapy. To overcome the above problems, finding a more suitable delivery approach for stem cells or EVs-based therapy should be the most direct and economical solution. A new administration

approach that could improve pharmacokinetics and could increase the tolerance of CNS disorder patients to multi-dose may help to better realize the therapeutic potential of stem cells or EVs-based therapy.

## 2.2. The potential of intranasal administration in CNS disorder treatment

In recent years, IN administration is getting increasing attention due to the unusual transport pattern<sup>34,82,83</sup>. Special histological features in some nasal cavity regions enable direct access of drugs to the brain<sup>84</sup>. The olfactory sensory neurons in the olfactory area of the nasal cavity and two branches of the trigeminal nerve in the respiratory region of the nasal cavity have free nerve terminals under the mucosa. Therefore, the drug can pass through the epithelium and reach the olfactory bulb or pons along the axons. Then the drug can enter the perivascular space of the cerebrovascular or spread with the cerebrospinal fluid, and finally, distributed throughout the CNS.

As the IN route could bypass the BBB and avoid the first-pass effect, IN route is used for drugs that have difficulties reaching a therapeutic concentration in the CNS, like protein drugs<sup>85-87</sup>. Pharmacokinetic studies have shown that although the nasal epithelium reduces the bioavailability of IN delivery, when the blood drug concentration is the same, the drug concentration in most areas of the CNS after IN administration is ~10 times higher than that of the systematic injection<sup>88-90</sup>. What's more, by increasing the IN-delivery dose, the drug concentration in each part of the CNS would increase proportionally. On the contrary, a study showed that increasing the arterial injection dose by 20-fold can only result in a 1.2-fold increase of the drug concentration in the CNS<sup>89</sup>. These results indicated that IN route may be more

feasible to achieve therapeutic drug concentrations in the CNS compared with systematic injection.

The drug delivery of IN route is non-invasive. IN-delivery devices like spray, atomization, nose drop, or other devices have relatively simple administrative procedures and would not cause extra harm to the patients<sup>91,92</sup>. Therefore, patients could tolerate high-frequency IN administration. Repeated IN delivery in a week, even in a day, is commonly found in clinical trials involved with IN administration, as presented in Table 2<sup>93–95</sup>. And with the assist of administration devices, self-administration is also available for patients, which reduces the influence of the frequent dosage on patient life. Therefore, IN route could enable flexible regimens that have different treatment courses and frequencies for CNS management.

The advantages of IN delivery in pharmacokinetics and therapeutic regimen guarantee its benefit in efficacy. IN delivery of nerve growth factors<sup>96</sup> (NGF), insulin<sup>97</sup>, oxytocin<sup>98</sup>, erythropoietin<sup>99</sup>, and other drugs have shown promising effects in various CNS disease models. Clinical trials using the IN route for drugs delivery are also emerging in recent years, Table 2 lists the representative clinical trials using IN delivery for CNS disorder treatment<sup>81,93–95</sup>. The IN delivery is applied on various clinical occasions and could be used for the elderly or children in clinical trials. Better prognosis and long-term relief were also observed in many clinical trials compared with intravenous administration.

Overall, IN delivering drugs could improve pharmacokinetic, increase patient tolerance and enable flexible multi-dose therapeutic regimens. However, there is less discussion about IN delivery of stem cells or EVs. If these advantages of the IN pathway can also be found when delivering stem cells or EVs, it may compensate for the problems in stem cells or EVs-based therapy. IN pathway would be a possible way to facilitate the clinical translation of stem cell and EV therapy.

### 2.3. The transportation process of stem cells or EVs in the intranasal pathway

IN route mostly was used for the delivery of drugs or proteins with a relatively small size<sup>34</sup> (less than tens of nanometers). The transport capacity of IN route for larger particles has been found only recently<sup>100,101</sup>. Epithelium in the olfactory and respiratory

region is the first barrier for large particles transportation. Researches showed in the olfactory epithelium, the resident basal cell would differentiate and replace sustentacular cells or olfactory sensory neurons. The ciliated and goblet cells in the respiratory epithelium also would undergo similar replacement<sup>102</sup>. The process of turnover of cells in the nasal epithelium results in discontinuous tight junctions and a higher permeability<sup>84,103</sup>. Furthermore, lipid nanoparticles were found could transiently open the tight junctions in the nasal epithelium, which means nasal epithelium is more permeable to particles with lipid surfaces<sup>104</sup>. Besides paracellular bypass the epithelial barrier, nanoparticles including lipid nanoparticles also could cross the epithelium through transcytosis<sup>105,106</sup>. EVs of stem cells have similar size and surface properties as lipid nanoparticles, a similar transport pattern to cross epithelium may be adapted to EVs. The inflammation tropism ability of stem cells or EVs is also important in the trans-epithelial migration. Stem cells or EVs could interact with epithelial cells and cause a cytoskeletal reorganization of epithelial cells, which makes stem cells or EVs capable to overcome the barrier<sup>107</sup>. This process is mediated by chemokine gradients and could be augmented under inflammation states. Researchers found the presence of stem cells or EVs in the sub-mucous space after IN delivery<sup>84</sup>, the number of cells or EVs would increase under pathological conditions<sup>108</sup>. These findings support the inflammation tropism of stem cells or EVs that could facilitate trans-epithelial migration.

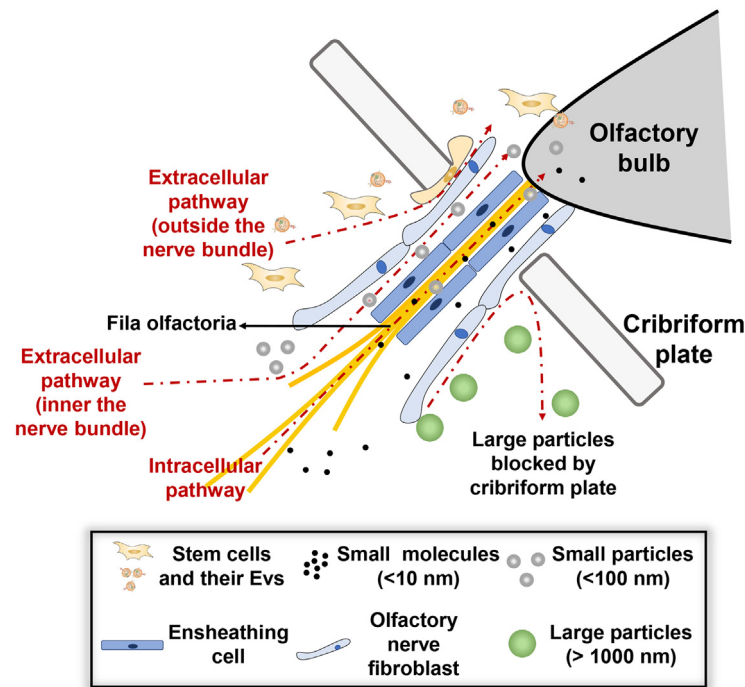
The cribriform plate is another barrier for nose-to-brain transportation through the olfactory nerve pathway<sup>84</sup>. The small molecular could cross the cribriform plate intracellularly by the axonal transport of olfactory sensory neurons (Fig. 1). And olfactory ensheathing cells in the olfactory nerve bundle maintain continuous open spaces for the regrowth of olfactory nerve fibers, creating an extracellular path (within the nerve bundle) to cross the cribriform plate<sup>34</sup> (Fig. 1). However, constrained to the diameter of the olfactory sensory neurons (~500 nm), both the intracellular and extracellular route seems not applicable to large particles. Researches showed intact polycaprolactone nanoparticles or chitosan-coated nano-emulsions with a size of ~100 nm could not cross the cribriform plate through the olfactory pathway<sup>109,110</sup>. Only loaded cargos could be released and enter the brain region<sup>110</sup>. Intact nanoparticles with a

**Table 2** Representative clinical trials using IN administration for CNS disease management.

Condition	NCT number	Phase	Intervention	Status	Ref.
Alcoholism	NCT03339024	Phase III	Repeated IN administration of oxytocin spray twice a day for 30 days	Completed	93
	NCT01829516	Phase IV	Single IN administration of oxytocin	Completed	N/A
Schizophrenia	NCT00575666	Phase IV	Repeated IN administration of insulin 4 times a day	Completed	N/A
Alzheimer's disease	NCT03245437	Phase IV	Oxytocin nasal spray	Completed	N/A
	NCT01767909	Phase II/III	Daily IN administration of insulin for 12 months	Completed	94
Treatment-resistant depression	NCT02497287	Phase III	Repeated IN self-administration of esketamine twice a week for 4 weeks	Completed	81
Ischemic stroke	NCT03686163	Phase IV	Daily IN administration of NGF for 2 weeks	Completed	N/A
Epilepsy	NCT01999777	Phase III	Single IN administrated of midazolam	Completed	95

Resource of the clinical trial is from [ClinicalTrials.gov](http://ClinicalTrials.gov).

IN, intranasal; NGF, nerve growth factor; N/A, not applicable.



**Figure 1** Possible pathways for stem cells, EVs, and particles of different sizes to pass through the cribriform plate. In the olfactory pathway, small molecular drugs could be endocytosed by the neuron and across the cribriform plate through intraneural transport. Small molecules also could enter the gaps in the olfactory nerve bundle and be extracellularly transported to the olfactory bulb. Although small particles (<100 nm) also could be endocytosed by the neuron, the extracellular pathway is more important for transportation, and the trigeminal nerve pathway (not shown) could be dominant. Large particles (>500 nm) could be found near the olfactory nerve bundle, yet intact particles could not reach the olfactory bulb, cribriform blocked the entry of large particles. But stem cells or EVs could cross the cribriform plate through an extracellular pathway independent of the olfactory bundle, the position where stem cells or EVs crossing the cribriform plate is adjacent to the olfactory bundle, the inflammation tropism ability of stem cells or EVs may play an important role.

size of  $\sim 100$  nm could be detected in the trigeminal nerve tract, and these nanoparticles could reach the brain stem through the trigeminal nerve pathway<sup>109</sup>. But as the size increases to  $\sim 1000$  nm, intact nanoparticles are no longer able to reach the brain stem through the trigeminal nerve pathway in a short time<sup>110</sup>. Therefore, it seems hard for stem cells (10–20  $\mu\text{m}$ ) and EVs (100–1000 nm) to reach the brain especially through olfactory nerve pathways. Yet a quick localization in the olfactory bulb of stem cells or EVs after IN administration was confirmed by many studies<sup>111,112</sup>. Using MRI tracking, a dynamic transfer from the nasal region to the olfactory bulb could also be observed<sup>18,41,111,113</sup>. These articles suggest that stem cells or EVs appear to travel along with the olfactory nerve bundles (Fig. 1). Detailed analysis showed the signal of stem cells is just adjacent to, but not within, the olfactory nerve bundles, which is different from the known olfactory nerve pathway<sup>84</sup> (Fig. 1). These findings imply stem cells or EVs may employ a novel route crossing the cribriform plate. Immune cells like T cells and monocytes could be observed crossing the cribriform plate from the CNS to the nasal region. When crossing the cribriform plate, immune cells are also found adjacent to the olfactory nerve yet not within the nerve bundle, just like stem cells entering the CNS<sup>114–116</sup>. Therefore, there is a hypothesis that stem cells or EVs may enter the CNS following the reverse route used by immune cells. The cytokine concentration gradients may drive the trans-cribriform plate movement of stem cells or EVs. Researches showed that increasing the concentration of SDF-1 (ligand of CXCR4) in the brain could increase the number of

stem cells entering the brain for several-fold, which supports the hypothesis<sup>108,117</sup>. The cerebrospinal fluid also exits from the brain through the cribriform plate and enters the nasal area unidirectionally, suggesting the hypothesis is histologically feasible<sup>118</sup>. Yet the pressure gradient between the nasal cavity and the CNS could be a barrier, the stem cells or EVs need to overcome the relatively higher pressure in the cerebral. Hence active migration ability may be necessary to enter the CNS through this hypothetical pathway, which explains why nanoparticles cannot enter the brain through this hypothetical pathway.

In a word, the inflammation tropism of stem cells or EVs play an important role in crossing the epithelium and cribriform plate barrier. Stem cells or EVs may have a unique transport pattern to reach the brain compared with small molecules and nanoparticles. The detailed mechanism of the transport route of stem cells or EVs still needs further exploration.

### 3. Benefits of combining stem cells or EVs-based therapy with intranasal route

#### 3.1. Intranasal route increased lesion site distribution of stem cells or EVs

Since insufficient lesion site delivery is a problem for stem cells or EVs-based therapy, there are growing studies trying to improve

the targeting ability of stem cells or EVs. Modification of stem cells or EVs such as hypoxic treatment<sup>119</sup>, iron oxide nanoparticle stimulation<sup>8,120</sup>, and C-X-C chemokine receptor type 4 (CXCR4) overexpression<sup>121</sup> was employed to enhance the CNS lesion site targeting and improve treatment effect in preclinical studies. Our study had shown that iron oxide nanoparticle stimulation could increase MSCs distribution in the cerebral ischemia site. Reduced mortality rate and improved motor recovery were observed in MCAO mice<sup>8</sup>. But the engineering of stem cells or EVs would increase the difficulties in quality control and the complexity in manufacture. In contrast, the IN route is a more straightforward solution. Table 3<sup>122-131</sup> lists the representative pharmacokinetic data of IN administration of stem cells or EVs.

Gliomas treatment have difficulties in realizing enough drug concentration at the tumor site<sup>132,133</sup>. IN delivery of engineered MSCs or NSCs both showed a selective accumulation at the tumor site and better tumor penetration (Table 3). The high distribution of engineered MSCs or NSCs at tumor site also helped to extend survival time in primary tumor tissue grafted rats<sup>16,117,134,135</sup>. Pretreated nasal cavity with methimazole, drugs could delay the nasal clearance and enhance epithelium penetration, further promoting tumor site delivery of stem cells. Studies have shown that methimazole treatment increased the oncolytic virus-loaded NSC distribution at the tumor site after IN delivery to 20% and

prolonged the survival time of rats<sup>18</sup>. The above studies showed that IN administration was effective to increased tumor accumulation and to boost the efficacy of stem cells or EVs treatment in brain tumors.

In an aged  $\alpha$ Syn transgenic PD model, seven days after administration, MSCs were found that accumulated in the striatum where the loss of dopaminergic (DA) neurons happened<sup>136</sup>. In a 6-OHDA induced PD model rats, IN delivery of human olfactory ecto-mesenchymal stem cells (OE-MSCs) show a comparable grafted rate and even better therapeutic effect in DAergic markers rescue and motor function improvement compared with direct injection into the striatum<sup>137,138</sup>. These findings proved IN administration could also achieve a high CNS delivery efficiency in PD models.

For other diseases without a specific lesion part like AD. After IN administration, EVs are distributed in various brain areas, not limited to the olfactory bulb<sup>41</sup>. The EVs seem firstly migrate to the immature olfactory sensory neurons and then migrate along with the axon to the CNS. The intrinsic inflammation tropism (homing) ability of EVs may promote EVs to pass through the epithelium and increase the delivery efficiency. The high graft rate of EVs in the brain help to achieve robust neuroprotection, remarkable neurogenesis, and rescue of memory deficits in *App/Ps1* transgenic mice<sup>41,79,139,140</sup>. Furthermore, IN administration of EVs also

**Table 3** Representative pharmacokinetic data of IN administration of stem cells or EVs in different models.

Model	Pharmacokinetic data	Ref.
AD model mice	EVs were detected in the brainstem and olfactory bulb firstly at 0.25 h and reaching a peak at 1 h.	41
PD mice	Stem cells were detected in the whole brain area 7 days after administration. The brainstem and olfactory bulb contain the most cells (~20 %, respectively).	79
PD model rats	Stem cells were detected in the whole brain region and most distributed in the striatum and substantia nigra 2 months after administration.	122
AD model mice	Stem cells were detected in the olfactory bulb, hippocampus, ventral and dorsal cortex, brain splits, thalamus, and cerebellum 4 months after administrations.	123
Neonatal brain injury model rat	24 h after administration, stem cells were detected in the corpus callosum, cerebral cortex, olfactory bulb, and hippocampus.	124
SCI model of rats	Stem cells were detected in the lesion site of the spinal cord 2 and 4 weeks after the administration. Compared with 2 weeks ago, the number of MSCs in the lesion site even increased 4 weeks after administration.	125
SCI model rats	Exosomes were mainly detected in the lesion part of the spinal cord at 24 h after administration. The number of exosomes in the spinal cord even exceeds the brain.	126
Neonatal brain injury model mice	Stem cells were mainly detected in the brain at 12 h after administration.	127
Stroke model mice	Exosomes were mostly detected in the lesion hemisphere 1 or 24 h after administration. Number of exosomes in the ischemic part increasing overtime.	128
Glioblastoma model mice	Stem cells were mostly detected at the tumor site at 6 h after administration. The number of stem cells reached a peak at 24 h and remained steady in 5 days.	111
Glioma model mice	Stem cells were detected in the tumor area at 1 h, and the number of stem cells significantly decreased on day 5 but remained steady by Day 11; distribution of stem cells in the brain of irradiated animals was 2.8 times higher.	117
Glioma model mice	Stem cells were detected in the brain at 2h, reached a peak at day 1, and slightly decreased by Day 6.	129
Glioma model mice	Stem cells were detected in the tumor area at 24 h but reached a peak at 120 h after administration when treated with methimazole and fibrin glue.	18
<i>Btbr T+Ipr3tf/J (Btbr)</i> mice	Exosomes were detected in the brain at 24 h after administration.	130
EAE model mice	Secretome of amnion-derived multipotent progenitor cells (ST266) was selectively accumulated in the optic nerve and vitreous at 30 min after administration.	44
Chronic alcohol consumption model rats	Exosomes were detected in the brain at 2 h and gradually increased within 24 h after administration.	131
Status epilepticus model mice	Exosomes were detected in the cortical and hippocampal at 6 h after administration.	14

AD, Alzheimer's disease; EVs, extracellular vesicles; PD Parkinson's disease; MSCs, mesenchymal stem cells; SCI, spinal cord injury; EAE, experimental autoimmune encephalomyelitis.

showed efficacy in aged *App/PS1* AD mice, whereas intravenous delivery failed to ameliorate symptoms<sup>79</sup>. The poor basic physical conditions account for the lack of efficacy in elderly mice, which is also one of the reasons for the failure of stem cells or EVs treatment in elderly patients. The better effectiveness of IN delivery in elderly mice suggests that IN route could better exhibit the curative effect of EVs-based therapy.

In cases of acute neuroinflammation disease where barrier function of BBB partly breaks down, stem cells or EVs could be detected in the lesion part within an hour after IN administration (Table 3)<sup>122–131</sup>. In a stroke model, the fast delivery of stem cells or EVs ameliorated post-ischemic events, a reduced infarct area and inflammation were observed, and junctions of vascular were well persevered after treatment<sup>141</sup>. In perinatal brain injury models, a similar fast delivery to the brain was also observed in rat pups. NSCs or exosomes of MSC reached the brain in hours<sup>124,142</sup>. The immediate intervention significantly rescued motor and cognitive development of pups who received stem cells or EVs-based therapy<sup>13,142,143</sup>. Even in spinal cord injury models where trauma is out of the brain, IN delivery stem cells or EVs still realized highly efficient lesion part delivery in 24 h, and comparable or even better efficacy as an intralesional injection could be achieved using IN delivery<sup>125,126</sup>. The quick entry of stem cells or EVs into the CNS enabled by IN administration could help to seize the treatment window<sup>144</sup>, which is quite meaningful for the prognosis of acute CNS disease like stroke. As IN administration is relatively simple to conduct, IN delivery of stem cells or EVs may serve as a strategy in first-aid, though the storage of stem cells or EVs is a problem.

In a word, IN route provide a direct entry to the brain IN for stem cells or EVs<sup>130</sup> as shown in Fig. 2. The inflammation tropism ability of stem cells or EVs could facilitate epithelium penetration and further increased CNS delivery. These findings suggest that compared with delivering chemical compounds or protein drugs, IN route showed even better ability to improve biodistribution when delivering stem cells or EVs.

### 3.2. Intranasal route prolonged retention of stem cells or EVs in the lesion site

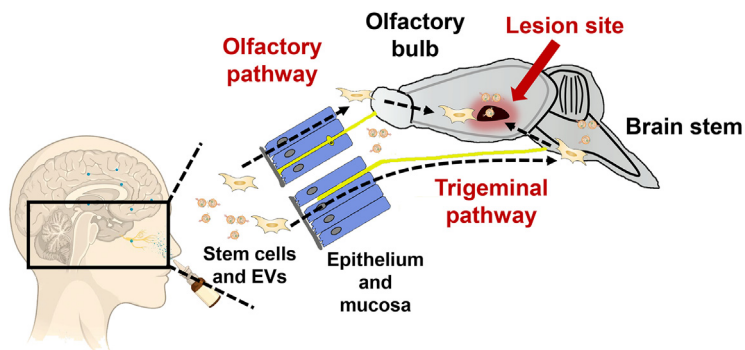
Embed stem cells or EVs in the scaffold is an effective way to protect the viability and prolong retention of stem cells or EVs. Our previous research<sup>12</sup> showed scaffold could significantly prolonged the retention of MSCs and EVs in the lesion site of SCI models. But the transplantation of scaffold needs surgery and is

not suitable for disease without a specific lesion site. Interestingly, stem cells or EVs grafted by IN route seemed to have longer retention in the lesion site<sup>18</sup>, as shown in (Table 3)<sup>122–131</sup>. IN route may also be a simple solution for the lack of retention.

IN route could prevent the damage to stem cells or EVs caused in syringe needles<sup>69,70,73,145</sup>. And stem cells or EVs would be directly transported into the brain but not immediately into the lesion site after IN administration, which also helps maintain the viability. In glioma models the number of stem cells in tumor site could remain steady or with mild decrease for in a week<sup>111,117,129</sup>. Methimazole treatment before administration, the signal of NSCs was even increase in 120 h after administration<sup>18</sup>.

Combination with radiotherapy also showed improved distribution and retention of MSCs, the number of MSCs at tumor site remained steady from Day 5 to Day 11 after administration<sup>108</sup>. As irradiation could upregulate the CXCL12 expression in tumor site, the inflammation tropism ability of stem cells may also involve in the improved retention. Similarly, in experimental autoimmune encephalomyelitis (EAE) models, IN delivery showed a superior in lesion site retention compared with intraperitoneal or intravenous injection and CXCR4 overexpression further enhanced retention of MSCs<sup>78,146</sup>. In 6-OHDA-induced PD model, stem cells or EVs were found to gradually accumulate to the striatum and substantia nigra<sup>147</sup>, stem cells or EVs could still be observed months after administration<sup>138</sup>. Meanwhile, the stem cells or EVs in other brain regions, like the olfactory bulb or brainstem, were constantly decreased after administration<sup>136</sup>. These findings proved that stem cells or EVs would continuously migrate from other brain regions (mainly the olfactory bulb) to the lesion site even weeks after administration. In SCI models, though the lesion site is far from the brain, the migration of stem cells is still found 4 weeks after administration<sup>125</sup>.

Therefore, the olfactory bulb may act as a reservoir for stem cells or EVs, as the olfactory bulb is the first stop for a major part of stem cell IN delivered. Stem cells or EVs in the olfactory bulb are slowly migrate to the lesion part in the CNS that helps maintain the number of cells or EVs in the lesion part<sup>111</sup>. And the inflammation tropism ability of stem cells or EVs seems to be the main driving force for the migration. In healthy mice or rats, stem cells or EVs delivered through IN route were found to linger in the olfactory bulb for a longer time and would have universal distribution in brain, lungs and liver, which also proved the reservoir function of the olfactory bulb<sup>126,130</sup>. These findings suggest the special transport pattern of IN route contribute to the longer retention (Fig. 3).



**Figure 2** Lesion site targeting of stem cells and EVs after IN administration. Stem cells or EVs migrate along the olfactory sensory neurons or trigeminal nerve and directly enter the olfactory bulb or brainstem. More stem cells or EVs could reach the CNS as these routes could bypass the BBB. Then they would gradually migrate to the lesion site in the CNS, achieving a higher distribution here.

In a word, IN route better preserved the viability of stem cells or EVs, and the olfactory bulb may serve as a reservoir that could sustain release of stem cells or EVs and maintain the number of stem cell and EVs in the lesion site. Hence, IN route could also be an answer for the insufficient lesion site retention in stem cells or EVs-based therapy.

### 3.3. Non-invasive procedures of intranasal route enable multi-dose and flexible regimen

There is a dilemma for CNS treatment: the less invasive intravenous route has unsatisfying lesion delivery; while high transplant efficient topical CNS injection is not tolerable by patients especially when repeated administration. In contrast, IN route could balance the delivery efficiency and patient tolerance. Therefore, multi-dosage of IN administration of stem cells or EVs could be the answer to the conflict, and it may be more effective and safer for CNS disease treatment.

Repeated IN delivery of MSCs and EVs caused no inflammation in the short-term; nor was there any behavioral abnormalities or histological changes in the long term<sup>148,149</sup>. Mouse<sup>148</sup>, rat<sup>43</sup>, and pig<sup>150</sup> pups of neonatal brain injury model showed good tolerance to IN administration of stem cells or EVs. A clinical trial also has used two doses of IN administration of  $5 \times 10^7$  MSCs to treat neonates suffering from perinatal arterial ischemic stroke (NCT03356821). Therefore, IN delivery of stem cells or EVs is patient-friendly and is tolerable even for infants.

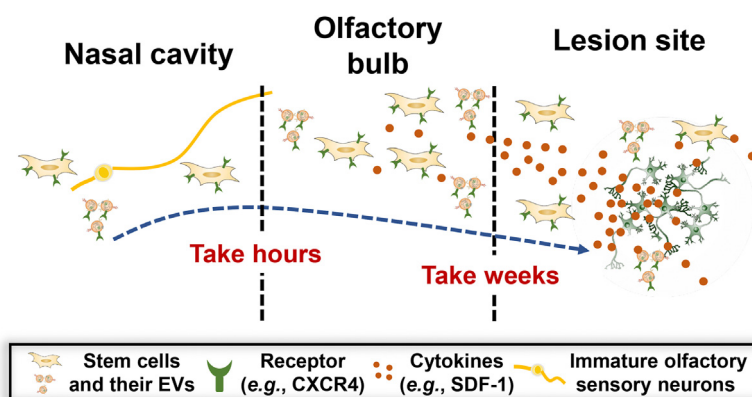
The more frequent dosage enabled by IN route appears to be beneficial for CNS disorder treatment (Table 4)<sup>151–153</sup>. In a perinatal asphyxia model, the additional dosage of MSCs secretome seven days after modeling dose seems further improve the locomotor activity, recognition memory, and anxiety of mouse pups<sup>152</sup>. In chronic diseases like neurodegeneration diseases, multi-dosage is more often used. The research found that AD model mice were well tolerated to weekly or even daily IN administration<sup>79,139</sup>. A single administration secretome of MSCs exposed to *App/Ps1* AD mouse brain homogenates (MSC-CS) through both IV or IN routes could reduce neuroinflammation in the brain and partly restore damaged memory. Yet, these functional recoveries are transient and have much less effect in 25-month-old aged mice. In contrast, weekly IN administration secretome of MSC-CS for four weeks led to a more long-lasting

improvement in memory for both young and aged AD mice. Increased neuronal density and prolonged lifespan were also observed in aged mice after multi-dose treatment<sup>79</sup>. However, the memory in treated mice could get impaired again after stop treatment for a month, which emphasized the importance of sustained and long-term intervention. Weekly administration of NSCs or repeated administration EVs of MSCs also showed long-term behavioral improvement in *App/Ps1* AD mice during treatment<sup>41,123,139,140</sup>. Therefore, for progressive diseases like AD, regularly IN administration of stem cells or EVs seems to be essential for long-term efficacy in AD treatment and the non-invasive IN route may be the best choice (Fig. 4A)<sup>79</sup>.

For PD treatment, pluripotent stem cells transplantation has been used to supplement the loss of DA neurons in substantia nigra pars compacta<sup>63</sup> (SNpc). Yet after a single administration, “on-off” reactions in treatment were still reported in some studies<sup>154,155</sup>. Multi-dose stem cell transplantation through the IN route may help to prolong the duration of the therapeutic effect, though there is no report on the use of IN for the administration of pluripotent stem cells. Besides pluripotent stem cells, researches showed that weekly IN administration of MSCs or EVs could reduce neuron loss and improve rat behavioral performance<sup>156–158</sup>. These reports support the feasibility of repeated IN administration of stem cells for PD treatment.

Psychiatric disorder patients are also needed long-term intervention. IN administration of MSC exosomes every other day for 8 days showed a long-lasting benefit in the social interaction of *Btbr*  $T^+Ipr^{fl/J}$  (*Btbr*) autism spectrum disorder (ASD) mice<sup>130</sup>. The research also suggested that the multi-dosage of IN delivered exosomes had similar efficacy as a single intracranial injection of MSCs<sup>159</sup>. Therefore, frequent repeated IN administration in a short period could also have a long-term effect, as indicated in (Fig. 4B)<sup>130</sup>. In the chronic alcohol intake model, the inhibition effect in ethanol intake began to decline 24 h after a single IV administration of MSCs-derived exosomes. Although single IN administration also only had a transient treatment effect, repeated IN administration within a month showed a more effective and long-lasting reverse in alcohol intake than single IV administration<sup>131</sup>.

In summary, long-term regularly administration of stem cells or EVs or shot-term multiple IN administration of them both showed an impressive effect in different animal models



**Figure 3** Migration track and timeline of stem cells and EVs. Most stem cells or EVs migrate adjacent to the immature olfactory neurons to the brain. Within a day, stem cells or EVs would mostly accumulate in the olfactory bulb. The cytokines in the lesion site (e.g., CXCL12) would gradually attract stem cells or EVs to the lesion site. The migration from the olfactory bulb to the lesion site could continue for weeks until most stem cells or EVs are concentrated in the lesion site. And the gradual migration to the lesion site could help to maintain the number of stem cells or EVs in the lesion site.



**Table 4** Representative treatment regimens using IN administration of stem cells or EVs based therapy.

Model	Treatment regimen	Pharmacodynamic data	Ref.
AD model mice	Once a day for 3 weeks	Pathology changes were ameliorated 2 days after treatment that is like a single intravenous injection of MSCs.	139
AD model mice	Once a week for 4 weeks	7 days after treatment, the memory was fully restored in aged mice after repeated IN administration. Single IN or intravenous administration fails to rescue memory fully.	79
AD model mice	Every two days for 2 weeks	Pathology changes were ameliorated and behavioral performances were improved at the end of treatment.	41
AD model mice	Once a week for 4 weeks	Behavioral performances were significantly improved 2 months after administration, and pathology changes were ameliorated 3 months after treatment.	123
PD model rats	Single administration	Behavioral performances were improved one week after administration and the improvement remained to 4 weeks.	147
Ischemic stroke and refusion model mice	1 h after modeling, twice a day for 7 days	Inflammation was inhibited and histological structure was restored at end of treatment. Behavioral performances were continuously improved during 7-days-treatment.	141
Neonatal brain injury model mice	A dose of MSCs administered 3, 10, or 17 days after modeling	Cognitive function improvement was achieved when administration at 3 or 10 but not 17 days after modeling.	151
Perinatal asphyxia model rats	Two doses of exosomes administered 2 h and 7 days after modeling	Inflammation was inhibited, and motor function was improved after the first dose; further improvement in behavioral performances was found after the second dose.	152
Neonatal brain injury model mice	Single dose administered immediately after modeling	Inflammation and brain tissue volume loss were inhibited, and behavioral performances were improved 2 days after administration.	143
SCI model rats	A single dose administered 24 h after modeling	Significant behavioral performances improvement was only observed at day 7 after modeling.	125
SCI model rats	2–3 days after modeling, once a day for five days	Behavioral performances were improved starting from 2 weeks after administration and a significant benefit was maintained to 8 weeks. Intralesional injection fails to improve behavioral performance.	126
Glioma model mice	Irradiation for 5 days combined with IN delivery of MSCs once a week for 4 weeks	The survival of mice was improved. Combination with irradiation could further enhance the efficacy of stem cell transplantation.	117
Glioma model mice	A single dose of NSCs administered after treating with methimazole and fibrin glue followed	The survival of mice was improved after stem cell transplantation. Methimazole and fibrin glue treatment could further enhance efficacy.	18
Chronic alcohol consumption model rats	Once a week for 5 weeks	Improvement in behavioral performances was achieved both after a single dose of IN or intracerebral exosomes, only repeated IN administration resulted in long-term relief.	131
Demyelination model mice	Once a week for 12 weeks	Pathology changes were ameliorated and behavioral performances improved 30 days after treatment.	146
<i>Bthr</i> mice	Every two days for 8 days	Behavioral performances were improved 2 weeks after treatment.	130

*(continued on next page)*

**Table 4** (continued)

Model	Treatment regimen	Pharmacodynamic data	Ref.
Schizophrenia model mice	Once a day for 14 days	Behavioral performances were improved 2–3 weeks after treatment.	153
EAE model mice	Daily administration for 4 weeks	Pathology changes were ameliorated, and behavioral performances were improved during treatment. Efficacy was diminished when treatment stopped.	44
SE model mice	Two hours after modeling IN deliver of two doses of exosomes within 18 h	Inflammation was inhibited, and long-term protection of memory and cognitive function were achieved.	14

AD, Alzheimer's disease; EVs, extracellular vesicles; PD Parkinson's disease; MSCs, mesenchymal stem cells; NSCs, neural stem cells; SCI, spinal cord injury; EAE, experimental autoimmune encephalomyelitis; SE, status epilepticus; IN, intranasal.

(Fig. 4)<sup>79,130</sup>. Since chronic CNS disease patients need long-term intervention, using IN administration would cause less pain and more negligible influence in patients' lives. The flexible treatment regimens provided by the non-invasive IN route also could solve problems in invasiveness and regimen of stem cells or EVs-based therapy.

#### 4. Future perspective for IN administration of stem cells or EVs-based therapy

As we discussed above, IN route showed many advantages over current used administration route: the IV route has a poor lesion site transplantation rate<sup>69–71</sup>; the topical injection is highly invasive and the frequency of dosage in topical injection was constrained<sup>77,145,160</sup>; other administration routes like intra-arterial (IA) or intraperitoneal injection (IP) also fail to realize high CNS delivery efficiency, and both are relatively invasive<sup>161,162</sup>. On the contrary, IN route could provide a direct nose-to-brain delivery and a high brain graft rate for stem cells or EVs. The non-invasive trait of IN route also makes it more suitable for CNS disorder patients who need long-term intervention. Table 5 shows the comparison among different administration routes.

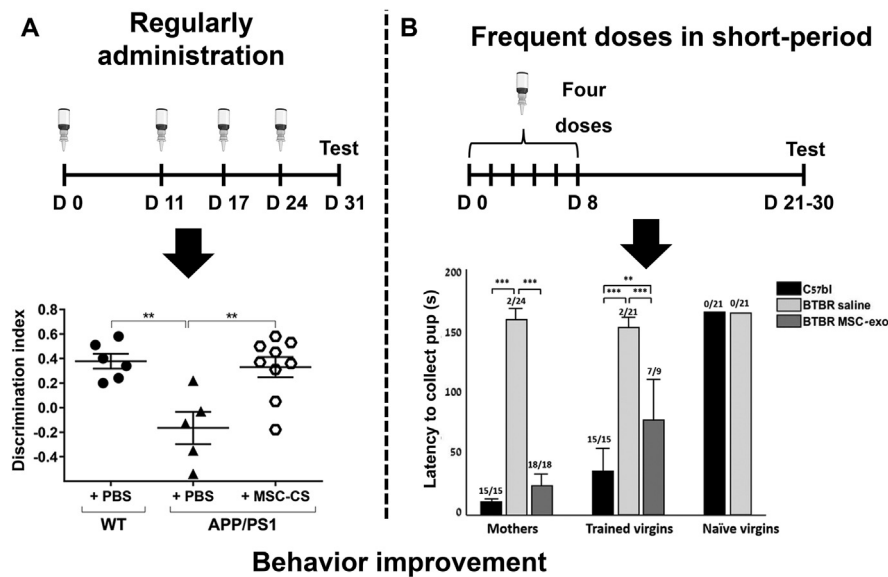
In recent years, several clinical trials have used IN administration of stem cells or EVs to treat CNS diseases, including PD, stroke, and neonatal brain injury. Table 6<sup>163</sup> lists representative clinical trials of stem cells or EVs delivered by IN route in recent years.

Nevertheless, the research on IN delivery of stem cells or EVs is still in its initial stage, and related researches still face some critical problems. The biggest problem is the relatively low bioavailability of IN route<sup>164,165</sup>. Currently, nose drops are primarily used in both preclinical and clinical for stem cells or EVs delivery. Stem cells or EVs would be cleared out of the nasal cavity before across the epithelium. The effective area for direct nose-to-brain delivery only takes up 3%–10% of the surface area of the nasal cavity, stem cells or EVs may enter circulation rather than enter CNS after crossing the epithelium. Therefore, only a part of stem cells or EVs could directly enter the brain.

The thermosensitive hydrogel could form *in situ* hydrogel after administration, reduce the clearance of the nasal cavity, fix stem cells or EVs in the olfactory region, and help increase the bioavailability of IN administration<sup>166,167</sup>. Currently,

chitosan<sup>168,169</sup> and poloxamer<sup>166,170</sup> are commonly used to prepare thermosensitive hydrogel. These materials show good biocompatibility and have been used in clinics. Hence the two materials could possibly aid the IN delivery of stem cells or EVs. Absorption enhancer is another strategy. There are a variety of compounds that could facilitate the absorption of IN administration. Among them, surfactants are an important category of absorption enhancers<sup>171,172</sup>. It is worth noting that both chitosan and poloxamer have been reported to enhance the permeability of epithelium<sup>173,174</sup>. Hence, chitosan or poloxamer thermosensitive hydrogel may also promote stem cell cells or EVs migration through the epithelium. Apart from surfactants, researches showed that pretreated nasal cavity with fibrin glue could inhibit cilia's movement and prevent the clearance of stem cells from the nasal cavity<sup>175</sup>. Hyaluronidase is another commonly used penetration enhancer for stem cells or EVs delivery. Hyaluronidase could degrade hyaluronic acid in the extracellular matrix (ECM) and loosening the epithelium, which help stem cells or EVs cross the epithelium barrier.

Another problem is that stem cells or EVs are unsuitable for the administration devices of IN delivery. IN-delivery devices can reduce drug loss during administration, promote deposition in correct region of nasal cavity, and enable self-administration. Currently, powder formulations or liquid formulations are primarily used in various IN administration devices<sup>176</sup>. For example, the Bi-Directional™ Breath Powered® device of OptiNose company is used for drugs like oxytocin powder delivery (NCT02414503), LMA MAD Nasal™ of Teleflex company is used for dexmedetomidine liquid delivery (NCT02955732). Yet, stem cells or EVs may lose their biological activity during preparation, storage, or delivery of formulations. The current formulation preparation process needs to be modified, or new formulation are required to help stem cells or EVs adapt to the IN-delivery devices. Biomimetic mineralization is a possible strategy to protect stem cells. Artificial mineral coats would form a rigid and degradable inorganic shell on the cell surface after mineralization and increased the resistance of cells to the adverse environment<sup>177</sup>. This technology may enable the preservation of stem cells in powders. Prepared stem cells into spheroids also could prolong the storage time. The research found that MSCs in spheroids could remain >90 % viability under ambient conditions and still showed therapeutic effects in mouse colitis models<sup>178</sup>. Since EVs of stem cells are not living cells, they may be easier to



**Figure 4** Different treatment strategies of IN administration. IN route could make flexible multi-dose treatment regimens possible. (A) Regular administration for long-term could maintain the behavior benefits in AD mice, the novel object recognition memory test showed the memory of aged AD mice fully recovered after treatment. Data are presented as mean  $\pm$  SEM (WT + PBS  $n = 6$ ; APP/PS1 + PBS  $n = 5$ ; APP/PS1 + MSC-CS  $n = 9$ );  $**P < 0.01$ , One-way ANOVA. Reprinted with the permission from Ref. 79. Copyright © 2020, The author (s). (B) Frequent doses in a short period also could bring a long relief. Improvement in learning of maternal pup retrieval behaviors and maternal pup retrieval were observed in autism spectrum disorder mice weeks after last administration. Data are presented as mean  $\pm$  SEM ( $n = 7$ );  $**P < 0.01$ .  $***P < 0.001$ , One-way ANOVA. Reprinted with the permission from Ref. 130. Copyright © 2018, The author (s).

be prepared into powder formulations. Lyophilization with cryoprotectants could avoid aggregation of EVs and extend the storage time of EVs<sup>179</sup>. Mannitol<sup>180</sup> and trehalose<sup>181,182</sup> were effective cryoprotectants for EVs and may enable EVs to adapt to powder IN-delivery devices.

The *in vivo* animal models for the studies of intranasal delivery is also a problem. For future research on stem cells or EVs formulations or administration devices, evaluation of pharmacodynamics and pharmacokinetics in animal models is necessary. Rodents like rats and mice are the most commonly used animals for experiments, as rats and mice are genetically similar to humans and have similar pathological mechanisms to humans in many diseases. However, the histological structure of the rodent nasal cavity is very different from that of humans<sup>34</sup> (Fig. 5). Rats and mice are obligate nasal breathers and have a more acute sense of smell. Therefore, the structure of the nasal passages of rats and mice is more complex and has a significantly higher surface area to volume ratio. The olfactory area of rats and mice accounts for 40%–50% of the total nasal surface area, while the human olfactory area accounts for only 10 % of the nasal surface area<sup>183,184</sup> (Fig. 5). Mucociliary clearance is also different between rodents

and human. The direction of mucociliary movement in human is backward towards the nasopharynx, opposite to the direction of mucociliary movement in rodents<sup>185</sup>. Furthermore, rodents have small nostrils, making the IN-delivery process cumbersome, and it's also difficult for rodents to adapt to IN-delivery devices. These facts make the pharmacokinetics of drugs delivered through IN route quite different between rodents and human. Therefore, data from large animals are crucial for preclinical studies and IN formulation and device development. Monkeys have a nasal cavity structure closer to humans<sup>34,186</sup>. Administration devices such as atomizers are also have been used in monkeys<sup>187</sup>. But the experimental monkey is very difficult to obtain, and the cost could be unaffordable. Rabbit may be an ideal substitute for large animals. The ratio of rabbit olfactory region in total nasal cavity surface is  $\sim 15\%$ <sup>188</sup> (Fig. 5), which is close to human. Current research shows that compared with other animals such as sheep or mice, the bioavailability and absorption rate of the drug in rabbits after IN administration is closer to that of humans<sup>189,190</sup>. Therefore, in addition to research on small animals, it is also recommended to conduct preclinical research on the IN delivery of stem cells or EVs in large animals such as rabbits. Due to the vast difference in

**Table 5** Comparison among different administration routes for stem cells or EVs transplantation.

Route	Invasiveness	Delivery efficiency	Process complexity	Bioavailability	Dosage frequency
IV	++	+	+	+++	++
IC	++++	++++	++++	+++	+
IA	+++	++	++++	+++	+
IP	++	+	++	+++	++
IN	–	+++	+	+	++++

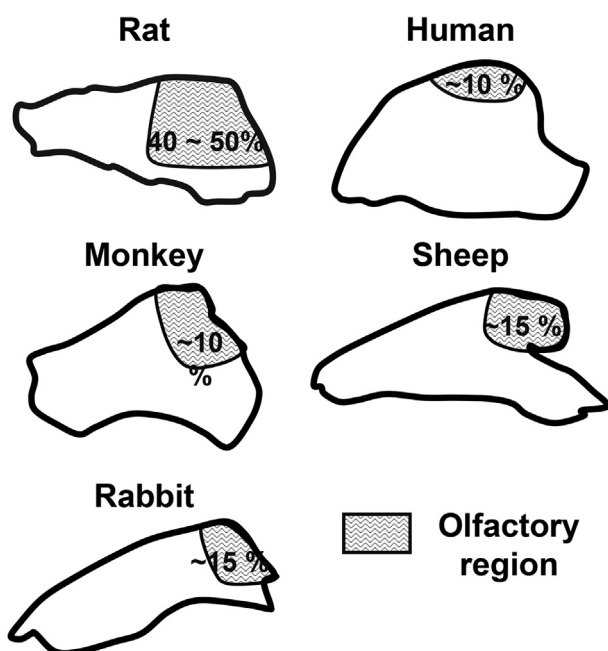
IV, intravenous; IC, intracranial; IA, intra-artery; IP, intraperitoneal; IN, intranasal.

**Table 6** Representative clinical trials of IN delivering stem cells or EVs for the treatment of CNS diseases.

Condition	NCT numbers	Phase	Intervention	Status	Ref.
Ischemic stroke	NCT05008588	Phase I/II	IN delivery of MSC's condition medium	Not yet recruiting	N/A
Perinatal arterial ischemic stroke	NCT03356821	Phase I/II	IN administration of MSCs at confirmation of the stroke and within the first week of onset	Completed	N/A
Alzheimer's disease	NCT03724136	N/A	IN administration of MSCs	Recruiting	N/A
Parkinson's disease	NCT04146519	Phase II/III	Tandem (IN + intravenous) injections of MSCs	Recruiting	163
Multiple CNS diseases	NCT03899298	Phase I	IN delivery of amniotic and umbilical cord tissue	Recruiting	N/A

Resource the clinical trial is from [ClinicalTrials.gov](https://clinicaltrials.gov).

MSCs, mesenchymal stem cells; CNS, central nerve system; IN, intranasal; N/A, not applicable.



**Figure 5** The nasal cavity structure of different animals and humans. The shaded area represents the olfactory region. The rodents like rats have a much larger olfactory region compared with human (50% vs. 10%). Nasal cavity of monkey is the one most similar to human. Other large animals like sheep and rabbit also have an olfactory region ratio similar to human.

the nasal cavity structure between rodents and humans, the data obtained on large animals will have more reference value for clinical translation.

## 5. Conclusions

Stem cells or EVs-based therapy brings hope to the treatment of various CNS diseases. Yet the clinical translation of stem cells or EVs is facing many challenges. From the recent clinical trials involved with stem cells or EVs-based therapy, we conclude 1) insufficient lesion site delivery, 2) retention and 3) patients' tolerance to multi-dose regimens are three problems in stem cells or EVs-based therapy. We suggest that utilizing IN route for the administration of stem cells or EVs could be a simple but effective solution. The feasibility of delivering stem cells or

EVs through IN path have been proved in many articles, though the detailed mechanism is not entirely clear. In current *in vivo* preclinical studies, we summarized that IN route could improve the biodistribution and retention of stem cells or EVs in the lesion site of the CNS. A more frequent and flexible IN treatment regime also enhances the efficacy of stem cells or EVs.

Yet current preclinical works are mainly focused on verifying the effect of IN delivery on the stem cell and EVs-based therapy for the CNS treatment. Future studies may also need to pay attention to the development of stem cells or EVs formulation for IN delivery. Integrate stem cells or EVs in formulation like hydrogel could prolong mucus retention and increase bioavailability. And these formulations may also help stem cells or EVs to fit IN-delivery devices, which could further simplify the administration procedure and improve delivery efficiency. And for the clinical translation, more experiments in large animals would be necessary, especially considering the vast difference between humans and rodents in the nasal cavity. Experiments on large animals would help understand the influence of formulation on the pharmacokinetics, as the data from large animal is closer to humans. Large animals are also more suitable for IN administration devices. Hence there is still a lot to be improved for the IN delivery of stem cells or EVs. Anyway, despite the intranasal route is still a new approach for the delivery of stem cells or EVs, we believe that it shows great potential and may become a critical factor in promoting the clinical translation of stem cells and EVs-based therapy.

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## Author contributions

Yaosheng Li collected related research article and wrote the manuscript. Honghui Wu revised the manuscript. Xinchu Jiang revised the manuscript. Yunfei Dong revised the manuscript. Juanjuan Zhen revised the manuscript. Jianqing Gao provided the

idea and revised the manuscript. All of the authors have read and approved the final manuscript.

### Conflicts of interest

The authors declare no conflicts of interest.

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