Procedures and Applications of Epidural and Intrathecal Injection

Subjects: Neurosciences

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Epidural and intrathecal routes are the most effective drug administration methods for pain management in clinical and experimental medicine to achieve quick results, reduce required drug dosages, and overcome the adverse effects associated with the oral and parenteral routes. Beyond pain management with analgesics, the intrathecal route is more widely used for stem cell therapy, gene therapy, insulin delivery, protein therapy, and drug therapy with agonist, antagonist, or antibiotic drugs in experimental medicine

epidural route intrathecal route drug delivery rats mice

1. Epidural and Intrathecal Injection Procedures

Epidural and intrathecal injections can be performed via acute needle puncture or catheterization. Acute needle puncture is usually used for single drug administration but can also be used for multiple doses [1], and intervals of 24 h, 2 days, and 7 days have been reported (**Table 1**). Acute needle puncture can be performed in anesthetized [2] or unanesthetized animals [1][3]; however, intrathecal injections in unanesthetized animals require greater skills. The intervertebral space of the spinal column is commonly used, but the lumbar region [2][4], especially L5–L6, is most commonly used for single acute injection [5][6]. However, multiple injections were performed through a single location, e.g., L5-L6 [1][7], L4-L5 [8], and top of the foramen [9] (**Table 1**). Proper needle insertion to the appropriate location and an accurate volume and concentration of the injectate, based on the species, are critical for the success and the recovery of animals; these are briefly discussed in **Table 1**, **Table 2**, **Table 3** and **Table 4**. For an effective IT injection via acute puncture, only one try is required; however, if the first effort fails, the needle should be removed, and a second attempt may be made. If the second effort fails, a different intervertebral space should be chosen [10]. In addition, shaving and aseptic preparation of the skin, needles, and administering agents are also important for success.

The exact positioning of a needle to the epidural space is difficult noninvasively and requires high skill because the distance between the epidural and intrathecal spaces is very small in rats and mice. Therefore, the applications of direct epidural injections using needle puncture in mice and rats are limited because the needle can easily penetrate the dura mater and enter the intrathecal space. Therefore, intrathecal injection by acute needle puncture [1][7][11], intrathecal catheterization [12][13][14], and epidural catheterization [15][16][17] but not epidural injection by needle puncture is frequently used in mice and rats.

1.1. Procedure of Intrathecal Injection by Acute Needle Puncturing

After administering anesthesia (e.g., volatile isoflurane), the thoracolumbar region is shaved. Needles are inserted through the intervertebral space where bony parts are absent, which is covered by the ligamentum flavum [18]. The angle of needle insertion varies but has been reported at over 20° [3][19][20][21], 45° [22][23], at 45° shown in videos but reported to be 70–80° in the discussion section [5], and at 70–80° shown in videos [11] in mice; an angle of 15–30° has been reported in rats [23]. Therefore, it is difficult to conclude what the optimal injection angle is (**Figure 3** and **Figure 4**). However, the authors of the current research also insert needles at angles of 70–80° and then reduce the angle to 30–45° during drug injection to easily spread out the injected drug from a narrow needle and to prevent CSF leakage during needle withdrawal (**Figure 4**). During insertion, when the tip of the needle reaches the bottom of the spinal canal (upper part of the vertebral body), the needle should not be pushed further as it may penetrate the intervertebral disc into the abdominal cavity [24] (**Figure 1** and **Figure 3**). The depth of needle insertion has been reported to be approximately 0.30 cm in rats after skin and muscle incision and 0.30–0.40 cm without skin and muscle incision in mice [22].

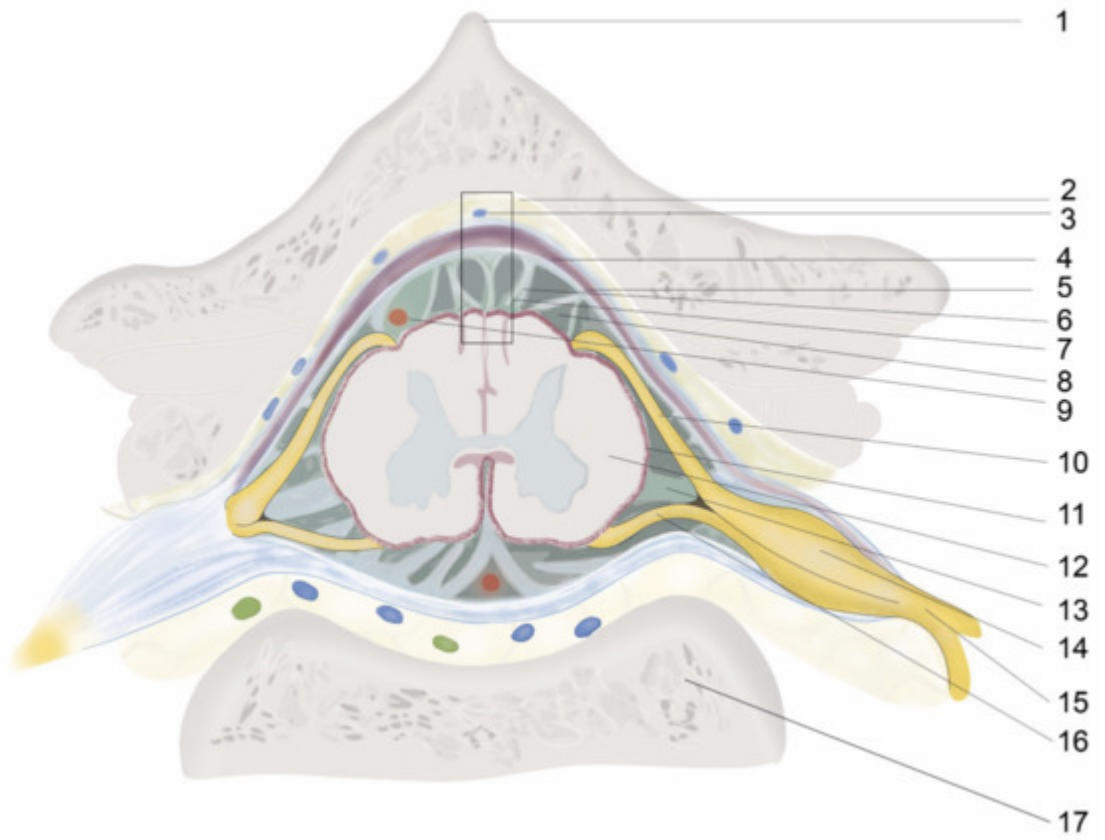

Figure 1. Illustration of cross-sectional anatomy of the spinal colum. 1. Spinous process, 2. Epidural space, 3. Intervertebral venous plexus, 4. Duramater, 5. Arachnoid mater, 6. Arachnoid trabeculae, 7. **Figure 2**, 8. Subarachnoid space and CSF, 9. Blood vessels, 10. Dorsal nerve root, 11. Pia mater, 12. Spinal cord, 13. Ligamentum denticulatum, 14. Dorsal nerve root ganglion, 15. Dorsal nerve root, 16. Ventral nerve root, 17. Vertebral body.

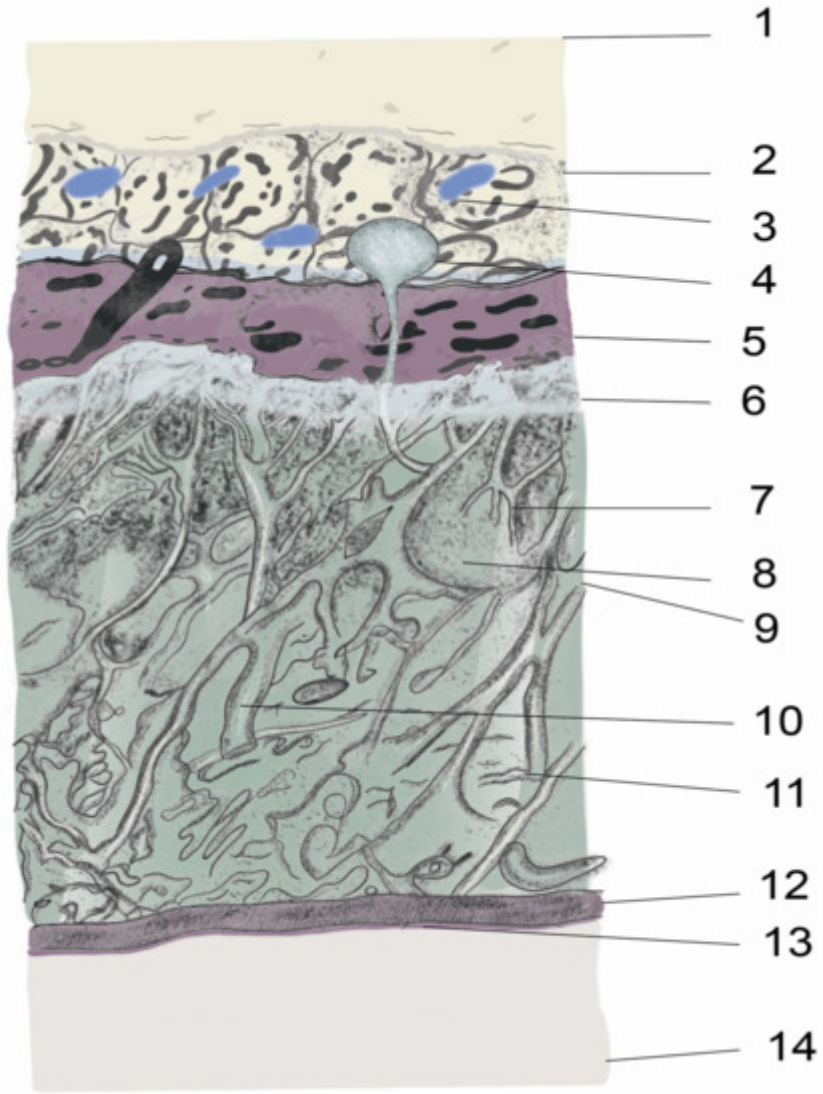

Figure 2. Illustration of anatomy of the spinal meninges and adjacent parts. 1. Ligament flavum, 2. Epidural space, 3. Intervertebral venous plexus, epidural fat 4. Arachnoid villi, 5. Duramater, 6. Arachnoid mater, 7. Arachnoid trabeculae, 8. CSF, 9. Subarachnoid space, 10. Major blood vessel, 11. Collagen fibrils, 12. Pia mater, 13. Glia limitans, 14. Spinal cord.

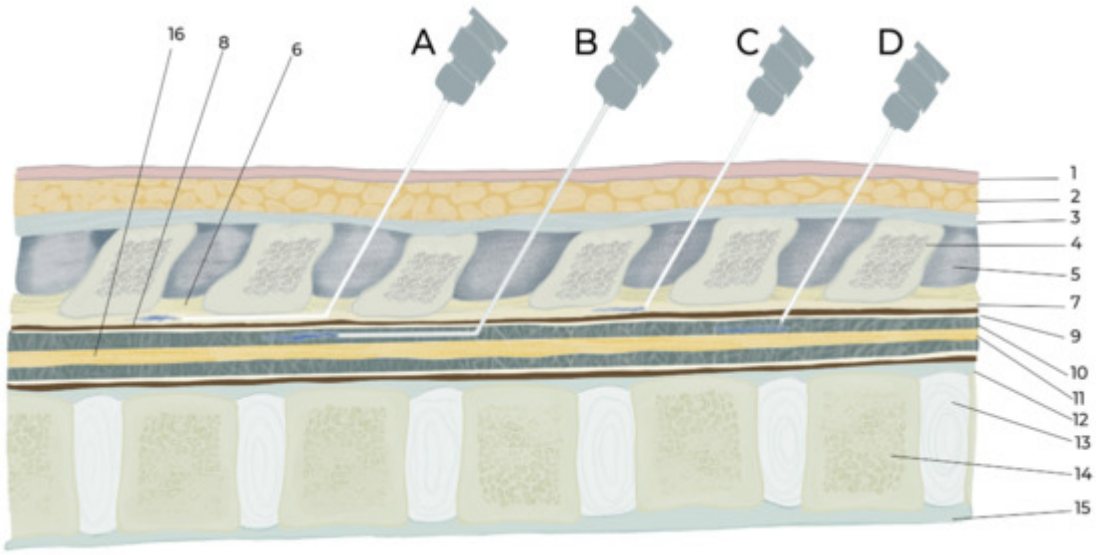

Figure 3. Illustration of intraspinal epidural and intrathecal drug delivery by catheterization and injection. A. Epidural catheterization, B. Intrathecal catheterization, C. Epidural injection, D. Intrathecal injection. 1. Skin, 2. Subcutaneous fat and muscle, 3. Supraspinal ligament, 4. Supraspinal process of vertebra, 5. Interspinous ligament, 6. Ligament flavum, 7. Epidural space, 8. Duramater, 9. Arachnoid mater, 10. Subarachnoid space, 11. Spinal cord, 12. Posterior longitudinal ligament, 13. Inter vertebral disc, 14. Vertebral body, 15. Anterior longitudinal ligament, 16. Spinal cord.

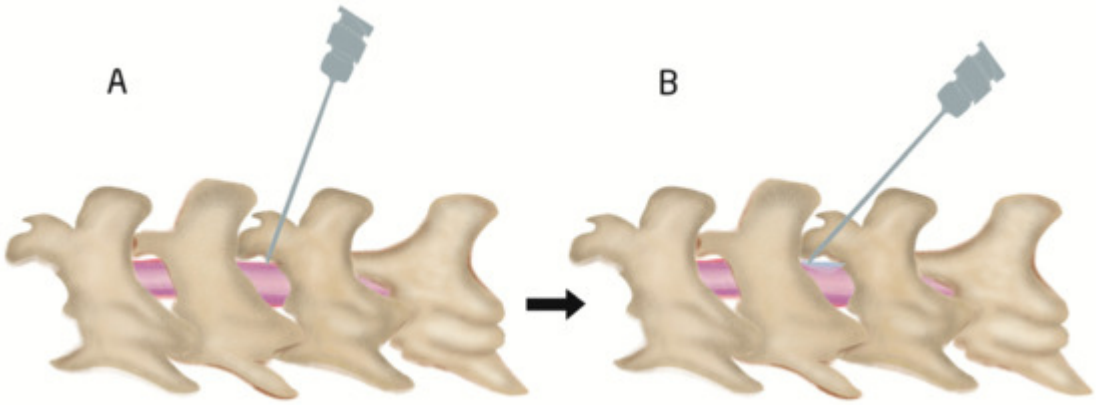

Figure 4. Illustration of intraspinal intrathecal injection site and angle. First insertion of needle at 70–80° angle (**A**) and then reduction until 30–45° (**B**) during drug injection to spread the injected drug easily and to prevent CSF leak out during withdrawing of needle.

The iliac crest and the L5–L6 intervertebral space are located by detection of the supraspinous process (**Figure 1**) of these two vertebrae (**Figure 5**). The pelvic girdle is then softly held by one hand to fix the dorsoventral position, and, on the other hand, a sterile needle is inserted at the appropriate angle to penetrate the ligamentum flavum and dura mater to reach the arachnoid space. However, some researchers use 1–3 cm longitudinal skin incisions over the spinous process of the desired intervertebral space in rats for better confirmation of the location during a

single acute intrathecal needle puncture [23][25][26]. The drug is then delivered slowly to allow for spreading. The delivery time varies (**Table 1**) and should be selected considering the characteristics of the injected solution and the species. In addition, after drug delivery, care should be taken to wait 15 s to 1 min before withdrawing the syringe (**Table 1**); otherwise, drugs may be pulled back into the syringe. Appropriate intrathecal positioning can typically be confirmed via the tail-flick test, but this response does not occur every time. Dura mater puncture can also be confirmed by other characteristics, such as the formation of an “S” shape by the tail, by hind paw retraction, and occasionally by backflow of the CSF [10][14][18]. After injecting a drug, temporary motor paralysis also occurs, which is a sign of successful drug administration.

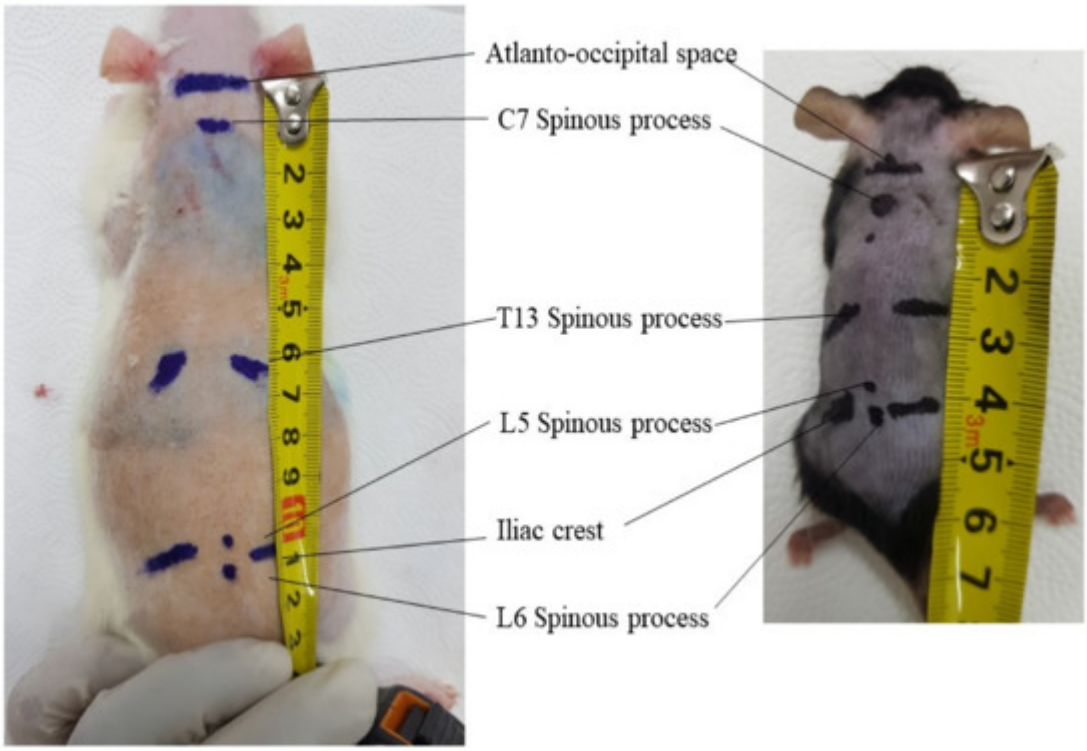

Figure 5. External location of antero-occipital space, C7, T13, L5, L6 spinous process, and iliac crest in 8 weeks old male Sprague Dawley rat and 8 weeks old male C57bl/6 mice. The average distance from antero-occipital space to L6 spinous process is 11 cm in rats and 4.2 cm in mice.

The injection volume is usually 5–10 µL in mice and 10–50 µL in rats, but different volumes have also been used (**Table 1**, **Table 2**, **Table 3** and **Table 4**). An intrathecal injection of 30–50% of the volume of the total CSF volume is defined as a larger volume [27]. After the thoracolumbar intrathecal administration of a larger volume, it is immediately transferred to the cere-bro-cervical region. Thirty-three and forty-two percent of the total CSF volume in a thoraco-lumbar intrathecal administration was reported as tolerable in humans and non-human primates, respectively [27]. However, the limits of safe injection volumes have not been characterized in mice and rats, and further experimental studies are needed. The total volume of CSF in mice and rats is 35–40 µL and 150 µL, respectively [28]. The needle size for intrathecal injection by needle puncture in rats and mice varies between articles, but a 30-G needle is commonly used for mice [1][29], and 25-G is used for rats [10][11] (**Table 1** and **Table 2**).

Furthermore, the dead space is sometimes ignored, but it has a significant impact on the accurate dosing of a drug because a very small total drug volume is injected via these routes. Dead space is the internal volume of the catheter or needle through which a drug passes from the syringe to the targeted region (e.g., epidural or intrathecal space). If the dead space is 10 µL and the injected volume is 10 µL, then the entire amount of the drug will remain in the catheter. To solve this problem, the dead space can first be filled with the injected agent, and the targeted volume should then be withdrawn in the syringe and then injected. Another method is by flushing with an equal volume of saline to replace the dead space occupied by the drugs [14][16][30]. However, care should be taken to prevent air bubbles when changing the syringe, which may cause adverse effects such as the alteration of subarachnoid pressure and injury to the nerves or meninges [31].

1.2. Epidural and Intrathecal Catheterization

Epidural and intrathecal catheterization are convenient and less stressful for animals when applying multiple doses for long-term medication. Intrathecal catheterization is administered in the atlanto-occipital and thoracolumbar regions [4][30], but epidural catheterization is only administered in the thoracolumbar region (**Table 2** and **Table 3**). Catheters are inserted into the epidural and intrathecal space either through the intraspinal space by penetrating/cutting the ligament flavum [4][18] or by laminectomy (e.g., dorsal laminectomy, lateral laminectomy) [18]. In addition, atlanto-occipital catheterization is conducted by penetrating the anterior atlanto-occipital membrane that joins the upper cranial border of the anterior arch of the atlas (C1) to the anterior inferior surface of the foramen magnum [13][30]. The catheter is usually extended from L4 to L6 until the lumbar enlargement region [12][32] because the spinal nerves of these regions have pain and motor function-related clinical significance for supplying the hind legs. However, in a spinal cord injury model, the target is to reach the injured area (e.g., from the atlanto-occipital ar-ea to the T10–T11 injured area) [33]. The length of the spinal column of rats and mice should be known before catheterization, and the length from the atlanto-occipital space to the L6 vertebra is approximately 11 cm in rats and 4.2 cm in mice (**Figure 5**). One study [34] showed that the length was consistently 11 cm in rats. Therefore, in atlanto-occipital catheterization, a catheter should be extended 8–11 cm in rats and 3–4 cm in mice (**Table 2** and **Table 3**). Therefore, care should be taken to avoid dura mater puncture in epidural catheterization and to avoid spinal cord injury in intrathecal catheterization. Usually, the length of catheter insertion for thoraco-lumbar catheterization in mice and rats is 1–2 cm but varies, with a maximum reported length of 4 cm [35]. The other end of the catheter is tunneled subcutaneously, and the opening is at the skin of the neck region, and the opening of the catheter is sealed or joined to a pump. Dura mater puncture can be confirmed based on behavioral responses such as those that occur with intrathecal injection. If animals exhibit any neurological deficits arising from the surgical procedure, they should be excluded from further experiments [12]. Additionally, 3 days after surgery, 10 µL of 2% lidocaine is often added to observe temporary motor paralysis and sensory loss for 20–40 min. If this does not occur, the animal should be excluded [14][21][21][30]. For epidural catheterization, animals should be excluded if CSF is aspirated or behaviors associated with dura mater puncture are observed [15] because epidural catheters should remain in the epidural space and above the dura mater where CSF is absent. Invasive epidural and intrathecal catheterization can be performed by direct observation of the dura mater by placing the catheter above the dura mater for epidural catheterization and by puncturing the dura and maintaining the catheter below the dura mater. Dead space should also be cautiously considered because of long catheters. PE-10 tubing is commonly used for

intrathecal and epidural catheterization in rats and mice (**Table 2** and **Table 3**). There are no studies showing how long catheters can be maintained after implantation. However, accurate placement of catheters was confirmed after 45 days in mice [36] and 9 months in rats [34]. Nonetheless, after experiments, the proper placement of catheters must be confirmed [21][36][37]. The injection site, injectate volume, concentration, frequency, duration, and purpose of epidural and intrathecal injections by catheterization are discussed in **Table 1**, **Table 2**, **Table 3** and **Table 4**.

Table 1. Intrathecal injection by acute needle puncture in mice and rats.

Species	Injection Site	Volume	Syringe Size	Time of Injection	Time of Syringe Withdrawal	Number of Injections	Reference
C57BL/6 mice	L4–5	10 μL	30-G needle to 50-μL Hamilton	3 μL/min	The needle was removed 1 min after completion and was kept in Trendelenburg position 5 min more	Single	[11]
C57BL/6 mice	L5–6	10 μL	30-G 0.5-in needle	-	-	Three injections, 24-h intervals	[29]
Kunming mice	L5–L6	10 μL		Delivered for more than 30 s	Syringe maintained for an additional 15 s to ensure diffusion before removal	Single	[7]
C57BL/6J mice	L5–6	5 μL	30-G in 10-μL Hamilton	-		Three injections at two-day intervals	[1]
FVB/NJ mice	L5–L6	8 μL	27-G needle 25-μL Hamilton syringe	1 μL/4 s	1 min after finishing delivery	Single	[5]
Mice	L5–L6	5 μL	30 G	1 μL/6 s	15 s	Single	[19]
C57BL/6 mice	top of the foramen magnum	20 μL	25-G, 1-mL syringe	Slowly	After 2 min	Three injections at 7 days intervals	[9]
CDI mice	L5–6	10 μL	30-G needle	-	-	Single	[38]

Species	Injection Site	Volume	Syringe Size	Time of Injection	Time of Syringe Withdrawal	Number of Injections	Reference
Mice		20 μL	30-G 1/2 in 50 μL Hamilton	Injections were delivered as a bolus within 5 s		Single	[39]
SD rat	L2–3	0.2 mL or 2 mL	1-mL syringe	1-mL syringe	After injection, rats placed upside-down at a 45° angle for 15 min	Single	[26]
SD rat	L5–6	30 μL	31 G		-	Single	[6]
Wistar rats	L4–5	15 μL	26 G	3 μL/min	-	Two injections, 24-h intervals	[8]
Wistar rats	L6–S1	0.02 mL/kg, average of 0.05 mL per	25 G	1 mL/min, average: 3 s/injection	1 mL/min, average: 3 s /injection	Single	[10]

Species	Site of Insertion	Catheter Size and Total Length	Inserted Length	Dead Space and Filling Agent	Injected Volume	Reference
Lumbar						
SD rat	L4–5	PE-10 (0.6 mm diameter)	1–2 cm	20 μL, saline	10 μL	[12]
SD rats	L4–5	PE-10 tube, 12 cm	2 cm	-	-	[4]
SD rats	L5–6	PE-10 (0.6 mm diameter), 10 cm	4 cm	-	10 μL	[35]
SD rats	L5–6	PE-10 (0.6 mm diameter), 15 cm	3 cm	4.5 μL, saline (7 μL)	10 μL	[14]
SD rat	L2 laminectomy, tip located between L3 and L5	SUBL-14	L3–L5	10 μL	25 or 50 μL	[40]
Rats	T13–L1	PE-5 catheters (outside	L2–L5	6 μL, PBS	20 μL	[36]

Species	Site of Insertion	Catheter Size and Total Length	Inserted Length	Dead Space and Filling Agent	Injected Volume	Reference
		diameter: 0.36 mm)				
Atlanto-occipital						
SD rat	Atlanto-occipital	ALZET catheter (PU-10 28G	8 cm caudally to reach lumbar enlargement	10 µL, sterile saline	20 µL	[30]
SD rat	Atlanto-occipital	PE-10	8.5 cm caudally to reach lumbar enlargement	10 µL, sterile saline	10 µL	[41]

Species	Site of Insertion	Catheter Size and Total Length	Inserted Length	Dead Space and Filling Agent	Injected Volume	Reference
SD rat	L4–5	PE-10 (0.6 mm diameter)	1–2 cm	20 µL, saline	10 µL	[12]
SD rat	T13–L1	PE-10	~3.0 cm until L5–6	100 µL of hyaluronic acid, 0.9% saline	100 µL of hyaluronic acid, 0.9% saline	[15]
SD art	T13–L1	PE-10 catheter	~3.0 cm until L5–6	10 µL of sa	160 µL	[16]
Mice	T11–T12	PU-10catheter	1 cm	-	50 µL	[17]

2. Uses and Application of Epidural and Intrathecal Injection

The epidural route is widely used for the induction of anesthesia in large animals and humans [42]. However, this route is widely used for analgesic purposes and not for anesthesia. In rats and mice, the intraperitoneal, intravenous, and intrarespiratory routes (i.e., for volatile anesthetics) are used to induce anesthesia. These routes are most commonly used for pain management with analgesics [41]. However, beyond pain management, the intrathecal route is widely used for stem cell therapy [8][9][38][43][44], gene therapy [11][29][44][45], delivery of immune cells [39], sedation [2], protein therapy, insulin delivery [46], mineral delivery [47], chemical delivery [15][47][48][49], and drug therapy using agonists [4][49][50], antagonists [4][40], antibiotics [47][51], and antiparasitic drugs [52] (**Table 4**). Intraspinal injection used in pain models can be divided into cancer pain models [44], including chemotherapy, induced pain models [1][52], and non-cancer pain models, such as models of arthritis [53], rheumatoid arthritis [49], diabetes-induced neuropathic pain [46][50], chronic pancreatitis-induced pain [54], spinal injury-induced pain [33][36], post-herpetic neuralgia [4], foraminal stenosis-induced pain [15][16], chronic DRG compression-induced pain [8], spared nerve injury [55], intrathecal capsaicin-induced spontaneous pain [48], chronic post-ischemia neuropathic pain [56], spinal nerve ligation-induced pain [6][12], and the acetic acid-induced writhing test [47] (**Table 4**). Furthermore, the intraspinal route is also used for the evaluation of safety and analgesic effects in normal healthy animals [41][57]. Beyond pain management, the intrathecal route is also used for drug administration for the

amelioration of spinal injury-induced spasticity [36] and the induction of itching and scratching in behavioral models [58] and a pruritis model [3] (**Table 4**).

Table 4. Different uses and applications of epidural and intrathecal injections.

Species	Method of Drugs Administration	Disease Model	Types of Agents Injected	Purpose of Injection	Concentration	Injected Volume and Vehicle	Reference
SD rat	ITc	Resiniferatoxin-induced postherpetic neuralgia	-Amiloride, a potent ASIC3 inhibitor -7,8-DHF, TrkB agonist, 3 mg/kg	-To evaluate involvement of ASIC3 and TrkB signaling in pain in dorsal root ganglia	100-µg amiloride daily for 7 days -3-mg/kg, TrkB agonist for 7 days	10 µL	[4]
SD rat	ITc	Spinal nerve ligation-induced pain model	Phosphodiesterase 4B-specific siRNA	-To reduce neuroinflammation	2 µg		[12]
SD rat	ITinj	Chronic pancreatis model	Cognate receptor C–X–C chemokine receptor type 4 (CXCR4) inhibitor	-to reduce pancreatic pain	5 µg/10 µL daily for one week	10 µL	[54]
SD rat	ITc	Freund's complete adjuvant-induced rheumatoid arthritis	Crocin	-To reduce rheumatoid arthritis-induced pain	100 mg/kg	20 µL	[49]
SD rat	ITc	Bone cancer pain model	Genetically engineered human bone marrow stem cells	-To reduce bone cancer pain	6×10^6 cells	10 µL	[44]
SD rat	ITinj	Neuropathic pain	Adipose tissue-derived stem cells (ASCs)	-To relieve neuropathic pain	1×10^6 cells	30 µL DMEM	[6]
SD rat	EDc	Foraminal stenosis-induced pain	Hyaluronic acid (HA)	-To relieve neuropathic pain	100 µL of HA	100 µL of HA	[15]
SD rat	EDc	Healthy rats	Gabapentin	-To evaluate safety and toxicity	30 mg	300 µL	[57]
SD rat	EDc	Lumbar foraminal	Polydeoxyribonucleotide	-To evaluate analgesic effect	0.1 mg/kg	160 µL	[16]

Species	Method of Drugs Administration	Disease Model	Types of Agents Injected	Purpose of Injection	Concentration	Injected Volume and Vehicle	Reference
		stenosis-induced pain					
Wistar Rat	ITc	Spinal cord ischemia	human umbilical cord blood stem cells	To improve spinal cord function	1×10^4 HUCBSCs	10 µL	[43]
SD rat	ITc	Spinal cord injury model	Embryonic Stem Cell-Derived Spinal GABAergic Neural Precursor Cells	To reduce central neuropathic pain and motor function	1×10^6 cells	-	[33]
Wistar rat	ITinj	Chronic DRG compression-induced pain model	Bone marrow stromal cell	-To reduce neuropathic Pain	1×10^6 cells	15 µL	[8]
CD1 mice	ITinj	CCI-induced neuropathic pain model	Bone marrow stromal cell	-To reduce neuropathic pain	1 or 2.5×10^5 cells	10 µL	[38]
Rat	ITc	Spinal cord injury-induced spasticity	-Potassium-chloride cotransporter KCC2 - BDNF	-To evaluate the involvement of KCC2 and BDNF in spasticity	20 µg 10 µg	20 µL	[36]
Rat	ITc	Phasic andincisional pain	Gentamycin, Streptomycin, Neomycin	-To evaluate Antinociceptive potency of aminoglycoside antibiotics	5 µg, 15 µg, 15 µg, respectively	10 µL	[51]
Rat	ITc	Diabetes-induced neuropathic pain	Insulin	-To evaluate Antinociceptive potency of insulin	0.2 U	10 µL	[46]
Rats, Mice	ITc	Diabetes-induced neuropathic pain	Sirtuin 1 agonist, SRT1720	-To reduce neuropathic pain	0.8 µg in rats, 1.4 µg in mice	10 µL	[50]
Rat	ITc	Spared nerve injury (SNI)	TMEM16A, U0126 inhibitors	-To find out the mechanisms of neuropathic pain	10 µg, 10 µg	10 µL	[55]
Mice	ITinj	Chemotherapy (Paclitaxel)-induced	-Artesunate	-To reduce chemotherapy-	100 µg	5 µL	[52]

Species	Method of Drugs Administration	Disease Model	Types of Agents Injected	Purpose of Injection	Concentration	Injected Volume and Vehicle	Reference
		neuropathic pain		induced neuropathic pain			
Mice		Neuropathic Pain	Decursin	-To reduce pain			[1]
Mice	ITinj	Spontaneous pain	Capsaicin	To induce spontaneous pain	0.5 µg in 10 µL		[48]
Mice	ITinj	PAR-2 activator trypsin-induced scratching behavior	-gastrin-releasing peptide (GRP) -Opioids	-To reduce scratching behavior	1 nmol/5 µL	5 µL	[58]
Mice	ITinj	Morphine-induced pruritis	Morphine	-To induce scratching behavior	0.3 nmol	5 µL	[3]
Mice	ITinj	Chronic post-ischemia neuropathic pain model	Human mesenchymal stem cells	-To reduce pain behavior	2×10^5 cells	5 µL	[56]
Mice	ITinj	Collagen-induced arthritis	ERK1/2 inhibitor (U0126), Tramadol, and NMDAR antagonist D-2-amino-5-phosphonovaleric acid	-To reduce pain behavior	1.6 µg, 250 µg, 0.5 µg, respectively	5 µL	[53]
Mice	ITc	Acetic acid-induced writhing test	Neomycin, gentamicin	-To evaluate antinociceptive effects	0.5–20.0 µg, 5–40 µg, respectively	10 µL	[47]

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