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Clarisa Peer, Editor

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Spontaneous Activity Directs Selection by Developing Visual Cortex Neurons

by Clarisa Peer

For nearly two decades, developmental neuroscientists have used ALZET pumps in their study of the intrinsic and extrinsic forces that direct the projection and topography of developing neurons. One area of intense study has been development of the mammalian visual system. Mammals can see at birth, having already developed a fully functional visual system. Studying neurons which terminate in the visual cortex, Catalano and Shatz recently reported the impact of spontaneous action potentials on neuron topography in a fetal feline model.[1]

In this unique model, which has been refined in numerous studies by Shatz and various co-investigators, fetal cats received an intracranial infusion of tetrodotoxin (TTX) during gestation. TTX is a neurotoxin known for its presence in Puffer Fish, a Japanese delicacy, and its use in voodoo zombification rituals. TTX specifically blocks voltage-gated sodium channels. When administered intracranially, it can prevent the generation of action potentials throughout the entire forebrain and midbrain.[2] This toxin was infused centrally by Shatz *et al.* via a brain cannula connected by catheter to a TTX-filled ALZET pump, Model 2002, which was sutured to the nuchal skin. Pumps were implanted during Cesarean section, and animals were returned to the uterus with pumps in place.[3,4,5] This method for TTX infusion directly to the brain parenchyma is critical because TTX does not appreciably cross the blood-brain barrier.

Catalano and Shatz timed the 2-week TTX infusion to occur at a point in gestation during which axons from the eyes have reached the dorsal lateral geniculate nucleus (LGN) and are extensively intermixed, prior to segregation into eye-specific layers.[1] During this period, axons from the LGN select the visual cortex as their target, a process thought to be initially independent of neuronal activity. However, these investigators found that blocking action potentials during this critical period altered the arrangement and accuracy of LGN projections.

Catalano and Shatz studied 25 fetuses, pairing littermates to receive an infusion of either TTX or citrate buffer into the

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diencephalon, about 2-3 mm above the optic chiasm. At the end of the infusion period, both retrograde and anterograde labeling of LGN neurons was performed. The number of axons extending into the visual subplate and cortex, the expected target sites, were counted. Also counted were axons extending into the auditory subplate and cortex, which are areas normally bypassed.

TTX-treatment appeared to divert LGN neurons from developing toward their target. These authors found that TTX-treated animals had fewer LGN neurons projecting into both the cortical and subplates of the visual cortex. (Figure 1) In addition, while LGN neurons did not appear to arrive at the auditory cortex, they were identified in this area's underlying subplate, which is normally bypassed. Many branches from LGN neurons were also identified in other non-visual areas along the pathway to the visual cortex. This work demonstrates that intracerebral TTX administration alters the spatial patterning of LGN projection, confirming a role for neuronal activity in refining thalamocortical connections.

For more information on the use of ALZET pumps for intracerebral infusions, TTX administration, or to study nerve development, please contact ALZET Technical Services.

1 Catalano SM, Shatz CJ. *Science* 1998; 281:559-562.

2 Campbell G, Ramoa AS, Stryker MP, Shatz CJ. *Visual Neurosci* 1997;14:779-788.

3 Shatz CJ, Stryker MP. *Science* 1988; 242:87-89.

4 Friedman S, Shatz CJ. *Eur J Neurosci* 1990;2(3):243-253.

5 Dalva MB, Ghosh A, Shatz CJ. *J Neurosci* 1994;14(6):3588-3

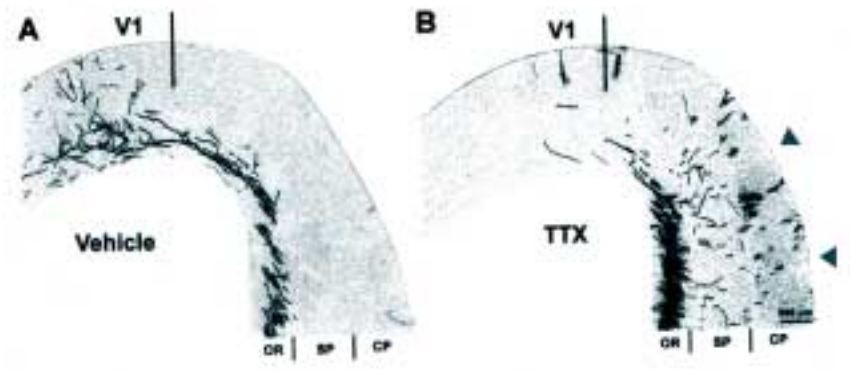

Figure 1. *TTX was infused intracerebrally via ALZET pump to block action potentials in the brain. Projections into the primary visual cortex (V1), the normal target for lateral geniculate nucleus (LGN) neurons, were significantly reduced in TTX- (B) but not vehicle-infused (A) animals as demonstrated by anterograde axonal labeling. In addition, TTX-infused animals had many branches extending into non-visual areas (B, see arrows) which are normally bypassed as seen in (A).*

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Intravenous Infusion in the Mouse Using ALZET Osmotic Pumps

by Jose R. Gadea

The development of "knock-out," transgenic, and new inbred mouse strains, along with increased animal welfare concerns and higher costs of maintaining larger laboratory animals, have encouraged the miniaturization of procedures and techniques. With this trend has come the need to develop a simple and reliable procedure for the intravenous (IV) infusion of agents to mice. Several methods for chronic IV infusion in the mouse have been proposed, but most have proven too troublesome to be widely accepted as practical methods for drug administration.[1,2,3] The major disadvantage of these protocols is that the mouse must remain attached to a tether or swivel during the infusion period, thereby restricting animal movement, preventing group housing of animals, and adding cost and complexity to the procedure. Additionally, these traditional externalized catheters are prone to failure and infection, which present a threat to animal welfare and data integrity.

ALZET osmotic pumps offer researchers a more practical means of chronic IV administration of agents in freely moving, unrestrained mice. Since the pumps are fully implantable, there is no need for external connections, allowing the animals to remain untethered and unrestrained during the entire infusion period. The lack of external connections helps minimize infection and may allow for the group housing of animals.

The use of ALZET pumps in mice for IV infusion is discussed in six publications. Other uses of ALZET pumps in mice are described in over 600 references. Seven of the ten ALZET pump models, ranging in duration from 24 hours to 4 weeks, are appropriate for use in mice. The smallest pump, which has a 100 µl drug reservoir, is suitable for subcutaneous (SC) implantation in mice that weigh at least ten grams. The 200 µl pumps are suitable for SC implantation in mice that weigh at least twenty grams, and not recommended for IP use. For IV infusion, the pump is implanted SC with the attached catheter leading into the vessel.

An information package containing surgical techniques, references, and tips for IV infusion in mice using ALZET pumps is available upon request from ALZET Technical Information Services. Additionally, the newly printed ALZET Technical Information Manual has detailed information on IV surgical techniques in rats, with mention of how to conduct such procedures in mice. A videotape which demonstrates surgical technique is also available free of charge.

1 Mokhtarian A, Meile M-A, Even PC. *Physiol Behav* 1993;54:895-898.

2 Barr JE, Holmes DB, Ryan LJ, Sharpless SK. *Pharmacol Biochem Behav* 1979;11:115-118.

3 Kelley BM, Bandy A-LE, Middaugh LD. *Physiol Behav* 1997;62(1):163-167.

Very Special Delivery

Pain Research and the Role of ALZET Pumps

by Lorri Perkins

Pain Etiology and Treatment

Pain is difficult to study and treat because of its subjective nature. Its treatment by physicians has been constrained by fear of addiction and strict controls on narcotics. It was not until the 1960s that the pathways of pain were elucidated in detail.[1] The World Health Organization's treatment plan for pain relies upon a variety of therapeutic options, including NSAIDs (non-steroidal anti-inflammatory drugs), compounds that are not normally considered to be analgesics (also known as adjuvants), non-opioids, and opioids. This paradigm for treatment begins with an NSAID for mild to moderate pain, combined with an adjuvant analgesic if needed. For moderate to severe pain, weak opioids such as codeine, hydrocodone, oxycodone, and propoxyphene may be prescribed. These may be combined with NSAIDs, adjuvants, or both. If analgesia is inadequate from this type of regimen, stronger opioids such as morphine, methadone, hydromorphone, levorphanol, or transdermal fentanyl are indicated.[1]

Neuropathic pain may be treated more effectively with adjuvants, which include tricyclic antidepressants, venlafaxine, or anticonvulsant drugs such as carbamazepine, phenytoin, or gabapentin. Sustained or controlled-release formulations have simplified continuous basal analgesia, which has improved the management of chronic pain.[1] New NSAIDs such as Celebrex® (celecoxib, Searle) and Vioxx® (rofexocib, Merck) are selective cyclooxygenase (COX)-2 inhibitors, which are indicated for inflammation or the short-term treatment of acute pain. By avoiding COX-1 suppression, this new class of NSAIDs may lead to fewer side effects, especially those involving the gastrointestinal tract.[2]

This recent emphasis on improved therapy for pain, and in particular neuropathic pain, has led to increased *in vivo* research on other types of drugs and also the etiology of pain itself. Recent drugs of interest include NMDA receptor antagonists, α_2-receptor agonists, and N-type calcium channel blockers such as SNX-111.[1] Indeed, the intrathecal infusion of SNX-111 into rats using ALZET pumps resulted in reversible and powerful antinociception, with minimal tolerance development.[3] In a recent effort to learn more about the etiology of pain, a group of researchers investigated axonal sprouting in the dorsal root ganglia after injury to a peripheral nerve.

Neurotrophin-Induced Sprouting in the Dorsal Root Ganglia after Peripheral Nerve Injury

In the dorsal root ganglia (DRG), peripheral nerve injury induces the sprouting of sympathetic and peptidergic terminals around large-diameter sensory neurons that project into the damaged nerve. It is thought that this sprouting may be involved in the chronic pain seen in patients with peripheral nerve injury. Zhou *et al.* from Finders University of South Australia investigated whether nerve growth factor (NGF) or neurotrophin-3 (NT-3) synthesis was upregulated in satellite cells that surround neurons in lesioned DRGs.[4]

NGF is a survival factor for developing sympathetic neurons, and also supports neurite outgrowth in culture and *in vivo*. After sciatic nerve injury, an increase in the level of NGF in DRGs has been confirmed by mRNA analysis and ELISA. Since sprouting occurs more extensively and quickly when the lesion is close to the ganglion, factors within the damaged ganglion may play a role. NT-3 is thought to have similar effects on sympathetic neuron development.[4]

Zhou *et al.* suspected that the satellite cells which ensheath the somata of peripherally-lesioned sensory neurons

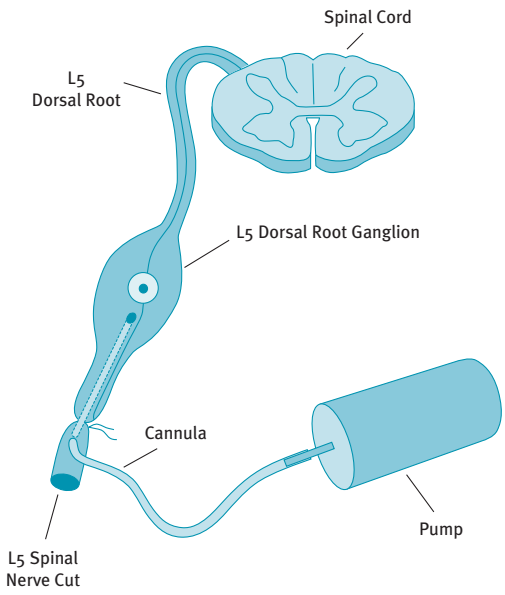


Figure 2. Antibodies were continuously delivered to the dorsal root ganglion for two weeks using a catheter connected to an ALZET Osmotic Pump.

synthesize NGF and NT-3. It may be the interaction between these factors and their low affinity receptor p75 that provides the chemotactic stimulus for sympathetic sprouting. This was tested by examining the gene expression of NGF and NT-3 in DRG after sciatic nerve ligation and transection. NGF and NT-3 synthesis was upregulated in satellite cells surrounding neurons in lesioned DRGs. This effect was substantial and sustained, as it was seen as early as 48 hours after nerve injury, and lasted for at least 2 months.[4]

Zhou *et al*. then infused agents into the ganglion. These included antibodies to NGF or NT-3, or normal sheep IgG used as a control. To accomplish this, an incision was made close to the ganglion on the epineural membrane of the rat L5 spinal nerve. Through this incision, a fine-tip catheter was inserted 3-4 mm into the nerve so that the tip lay within the ganglion (see Figure 2). This spinal nerve was then ligated so that the catheter was immobilized within the spinal nerve, and a suture was also placed in adjacent muscles to further anchor the catheter. The nerve distal to the ligature was then transected. The catheter tubing was connected to an ALZET osmotic pump implanted subcutaneously on the back of the animal.[4]

After two weeks of infusion, the DRG was analyzed for tyrosine hydroxylase (TH) immunohistochemistry, as TH is a marker for sympathetic neurons. While the number of tyrosine hydroxylase immunoreactive (TH-ir) axons within the ipsilateral DRG were dramatically increased with normal sheep IgG treatment, there was a substantial and significant decrease in the number of TH-ir axons in animals treated with NGF or NT-3 antibodies. NGF and NT-3 antibodies reduced sympathetic sprouting by respective values of 61% and 81% (ANOVA, $F_{3,21}$ = 13.19, $p < 0.01$), which is consistent with the involvement of locally synthesized and accumulated neurotrophins. (See Figure 3.)[4]

Zhou *et al*. concluded that the satellite cells which proliferate around axotomized sensory neurons express high levels of NGF, NT-3, and also their low affinity receptor p75. All sympathetic sprouts around sensory neurons were associated with p75-immunoreactive satellite cells in the L5 DRG after nerve lesions. Antibody delivery to the affected DRG blocked sympathetic sprouting, with the conclusion that satellite cell-derived neurotrophins are involved in the induction of sympathetic sprouting following injury to peripheral nerves.[4]

Similarly, Mannion *et al*. investigated the role of brain-derived neurotrophic factor (BDNF) in pain sensitivity. Using a different model, this group found that peripheral inflammation resulted in the substantial upregulation of BDNF mRNA and protein in the DRG in an NGF-dependent fashion. This inflammation also resulted in the novel expression of BDNF by DRG neurons with myelinated axons, and was prevented by anti-NGF. Mannion *et al*. concluded that BDNF played a role as a central modulator of tactile, stimulus-induced, inflammatory pain hypersensitivity.[5]

The work of Zhou *et al*. provides an elegant demonstration of how ALZET osmotic pumps can be used to create changes in the local environment. In this instance, continuous and targeted infusion elucidated mechanisms of neurosensory transmission. More information on the infusion of neurotrophins using ALZET osmotic pumps, and on the delivery of opiates, NSAIDs, antidepressants, and other treatments for pain, is available from ALZET Technical Information Services.

1 McGuire D, Luther RR, DuBois M. *Drugs of Today* 1998;34(5):481-486.

2 Koberstein W, Madell R. *Pharmaceutical Executive* 1999;19(8):42-54.

3 Malmberg AB, Yaksh TL. *Pain* 1995; 60:83-90.

4 Zhou X-F, Deng Y-S, Chie E, Xue Q, Zhong J-H, McLachlan EM, Rush RA, Xian CJ. *Eur J Neurosci* 1999; 11:1711-1722.

5 Mannion, RJ, Costigan M, Decosterd I, Amaya F, Ma Q-P, Holstege JC, Ji R-R, Acheson A, Lindsay RM, Wilkinson GA, Woolf CJ. *Proc Natl Acad Sci USA* 1999;96:9385-9390.

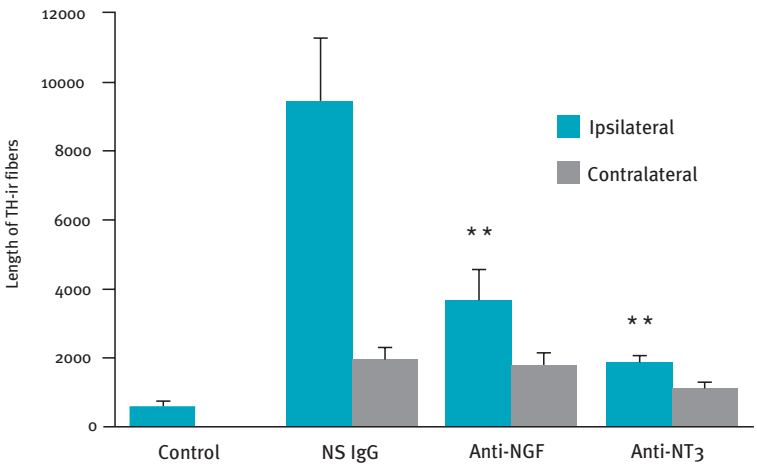


Figure 3. *Shown above are the effects of either locally infused antibodies to NGF or NT-3 or a control antibody, normal sheep IgG (NS IgG), on noradrenergic sprouting in dorsal root ganglia (DRGs) of rats with L5 spinal nerve lesions. As demonstrated by the decreased length of tyrosine hydroxylase immunoreactive (TH-Ir) axons, antibodies to NGF and NT-3 inhibited sprouting compared with the control group (NS IgG, ipsilateral DRG, **$p < 0.001$).*

Introducing the New ALZET Brain Infusion Kit II

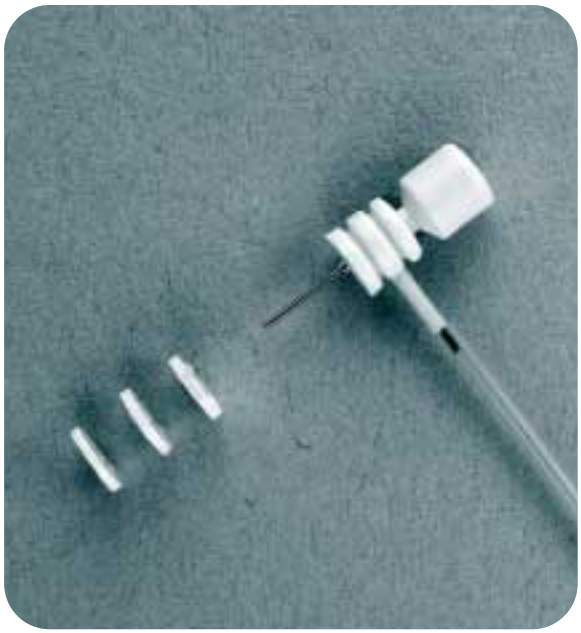


Figure 4. ALZET Brain Infusion Kit.

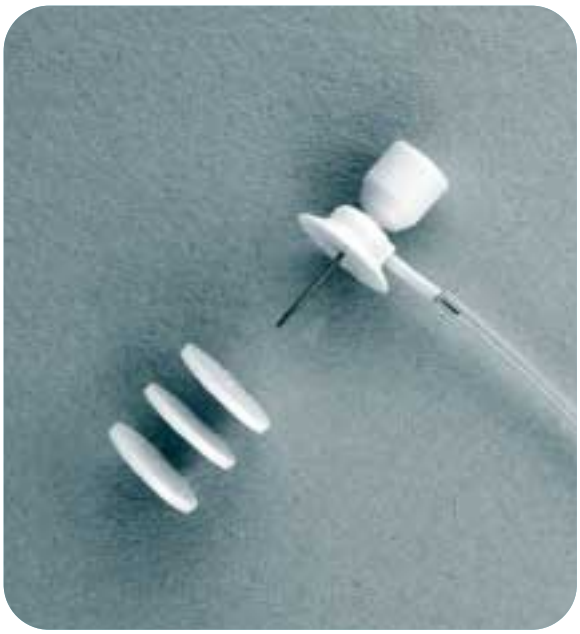


Figure 5. NEW *ALZET Brain Infusion Kit II.*

Evaluating the effects of a drug in the central nervous system requires that the agent penetrate the blood-brain barrier, and maintain sufficient concentration long enough to exert an effect. Over 900 published references demonstrate the successful use of ALZET pumps to administer compounds intracerebrally, either into brain tissue or the ventricles. Among these references, more than 30 used the original ALZET Brain Infusion Kit (Figure 4). This original kit was introduced in 1991. To facilitate infusion of compounds into the brain, the new ALZET Brain Infusion Kit II (Figure 5) offers a novel brain cannula specially developed for its stability when affixed to the cranium.

The design of this new cannula may eliminate the need to use a stabilizing screw. In addition, the low profile of the cannula allows for easy closure of the skin after placement. The cannula is placed stereotaxically through a hole drilled in the cranium, and connected by catheter tubing to an ALZET pump, which is placed subcutaneously on the back of the animal. Any model of ALZET pump, purchased separately, can be used with the kit.

Both the original ALZET Brain Infusion Kit and the new ALZET Brain Infusion Kit II include a 28 gauge cannula, which is 5 mm in length and is designed to target the lateral ventricle of a 250 gram Sprague-Dawley rat. Each kit includes cannulae and vinyl tubing sufficient for ten brain infusions, along with complete instructions. Also included are depth-adjustment spacers which can be attached to the cannula to shorten its penetration into the brain in 0.5 mm increments. The entire kit is sterile, having been exposed to a sterilizing dose of Co^{60} irradiation.

More information about the new ALZET Brain Infusion Kit II and the original ALZET Brain Infusion Kit is available on our web site at www.alzet.com. (Select "Products" and then "Brain Infusion Kits.") You may also contact ALZET Technical Information Services at 1-800-692-2990 or by e-mail at alzet@alza.com.

The use of ALZET Osmotic Pumps during MRI or NMR Studies

by Jose R. Gadea

Magnetic resonance imaging (MRI) is a noninvasive imaging technique used to produce high-resolution, computerized images of internal body structures for the detection, localization, and assessment of disease and tissue damage. Magnetic resonance images are obtained by placing the anatomical area of interest within a powerful, highly uniform, static magnetic field. For this reason, MRI requires that no ferromagnetic materials be present during the procedure, as metallic objects can cause poor image resolution and can be attracted to the magnetic source. This presents concerns for investigators planning to use ALZET osmotic pumps during MRI studies, since the flow moderator of the pumps includes stainless steel. However, ALZET pumps can still be used during MRI procedures when the stainless steel flow moderator is replaced with Teflon tubing of the same length and outer diameter as the original flow moderator. The Teflon tubing should have an outer diameter of 0.030 inches and an inner diameter of 0.013 inches, and is available for purchase from Scientific Commodities Inc. (catalog #BB311-30).[1]

In addition, researchers may be interested in obtaining magnetic resonance images of the brain while infusing agents through a brain infusion cannula. When such an experimental procedure is required, a non-metallic brain infusion cannula must be used. Cannula systems compatible with ALZET osmotic pumps and made with non-metallic components, such as plastic, Teflon, or fused silica, may be purchased from Plastics One, Inc.[2]

The following publication offers some details on the use of ALZET osmotic pumps during MRI:

Carpenter TA, Hall LD, Hogan PG. Magnetic resonance imaging of the delivery of a paramagnetic contrast agent by an osmotic pump. *Drug Design Delivery*. 1988; 3:263-266.

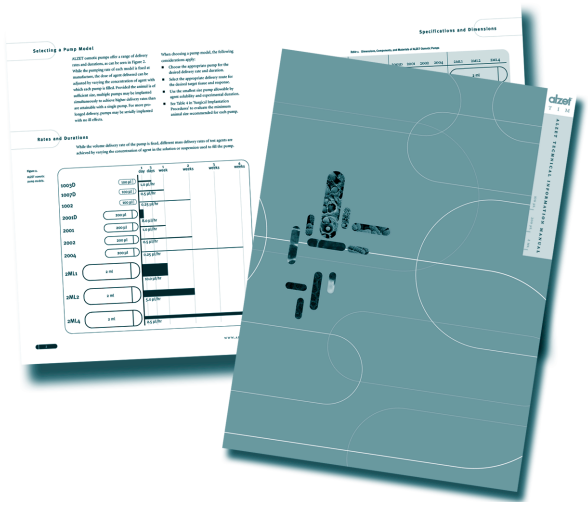
1 Scientific Commodities Inc., 1799 Kiowa Ave, Suite 107, Lake Havasu City, AZ 86403. (800) 331-7724, (520) 855-0159, or (520) 855-0993 *fax*.

2 Plastics One, Inc., 6591 Merriman Road SW, Roanoke, VA 24018. (540) 772-7950, (540) 989-7519 *fax*.

New ALZET Technical Information Manual Available

Recently, the ALZET technical information manual and catalog was updated with new information. This includes:

- an example of how to calculate the concentration of drug to use in the pumps
- information on the new Brain Infusion Kit II
- reference lists available from ALZET Technical Services
- information on protein and peptide delivery using ALZET pumps
- considerations to weigh when selecting a pump model and route of administration
- more detailed intravenous surgical procedures for rats, with additional tips for working in mice
- tips for performing brain infusion studies in mice



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Year 2000 Calendar Available

When planning your research schedule this year, consider using the new ALZET desk calendar. This calendar comes in a plastic case which doubles as a calendar stand, and incorporates a futuristic and technical look.

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ALZET Technical Information

For technical information about ALZET osmotic pumps, or for a complimentary custom search of our extensive bibliography, please return the attached business reply card or contact us:

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New Agents in the ALZET Literature

For over 20 years, the ALZET Osmotic Pump bibliography has been a valuable resource for scientists seeking to improve the *in vivo* delivery of therapeutic agents. We update our database periodically with the latest citations referencing the successful use of ALZET pumps in research studies. Our database now comprises nearly 6000 references. The following table lists some of the new agents recently added.

Agent	Therapeutic Category
(S)-HPMPA	Antimalarial
25-Hydroxycholecalciferol	Calcium regulator
A-779	Angiotensin-(1-7) antagonist
Amantadine	Antiviral, Antiparkinsonian
Antibody, anti-IL-8 (human)	Immunologic
Atenolol	Antihypertensive
B1 (dsFv)-PE38	Immunotoxin
Bay x 3702	5-HT_{1A} receptor agonist
BQ 788	Endothelin receptor antagonist
Buthionine sulfoximine	GSH synthesis inhibitor
Canrenoate	Aldosterone antagonist, Diuretic
Carmustine	Antineoplastic
Ceruletide	CCK analog
CNP-22 (C-type natriuretic peptide)	Angiotensin II receptor antagonist
Dipyridamole	Vasodilator, Anti-inflammatory
DMPPO	Phosphodiesterase type 5 inhibitor
DTPA	Chelating agent
Duloxetine	Antidepressant
Eprosartan	Antihypertensive
F-314	Oxytocin analog
Imidapril	Antihypertensive
Ketorolac	NSAID
L-365,260	CCK receptor antagonist
MKT-077 (also known as FJ776)	Antitumor agent
MZ-4-71	GH-releasing hormone inhibitor
Nandrolone decanoate	Anabolic steroid
PBA (4-Phenyl-3-butenoic acid)	Anti-inflammatory agent
PD 128,907	Dopamine agonist
PSC 833	Cyclosporin A analog
Quinacrine	Antimalarial, Anthelmintic
RES-701-1	Endothelin receptor antagonist
RS-33295	P-Glycoprotein inhibitor
ZD-7155	AT_1-receptor antagonist

References on these and other agents are available to you as a complementary service. Call the ALZET Technical Services Department at (800) 692-2990, or e-mail us at alzet@alza.com to request a free bibliography search customized to your area of interest.

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