Principles and Application of Stereotaxic surgery system

2013. 12. 30

Jee Geon Lee
UNIST Central Research Facilities (UCRF)

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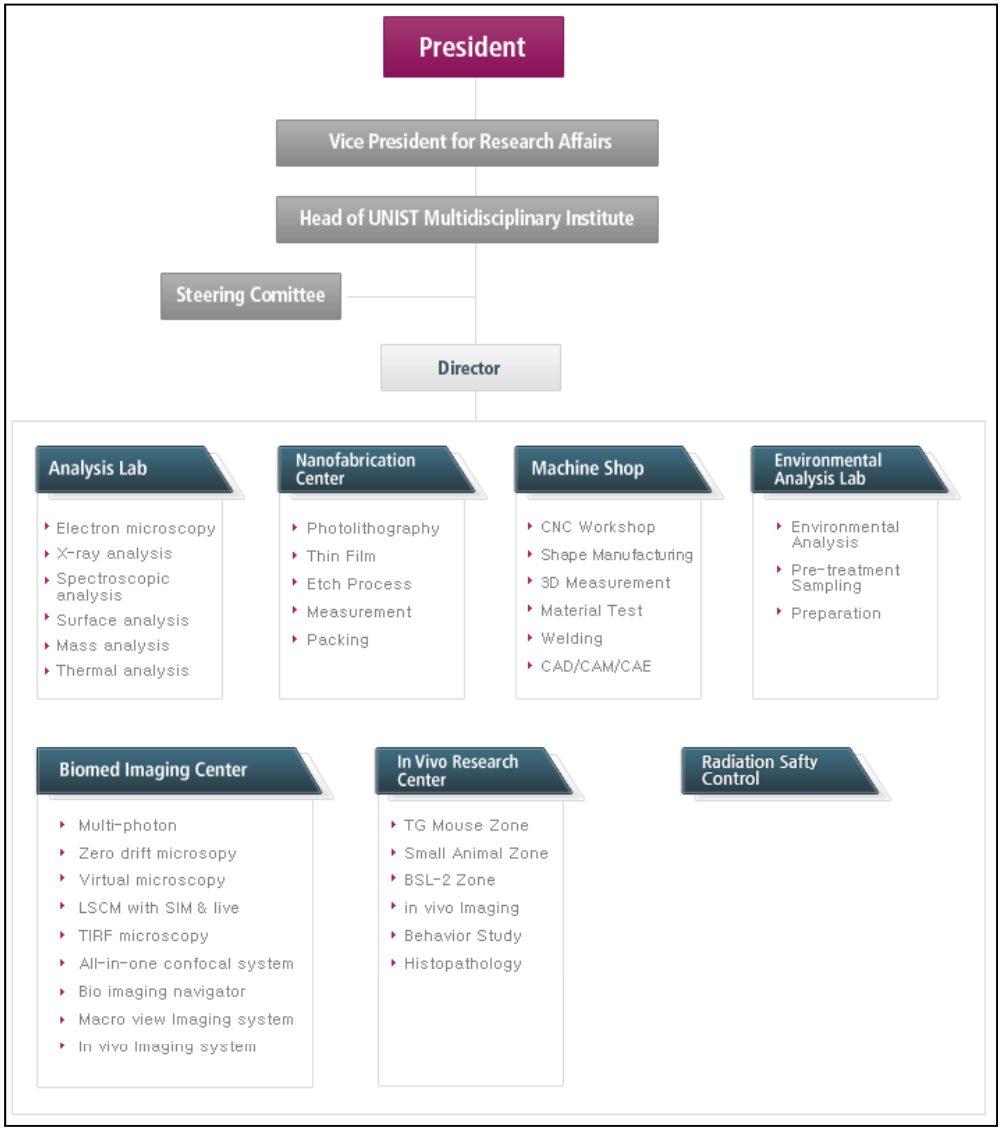
Organization of UCRF



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Process of animal zone & equipment

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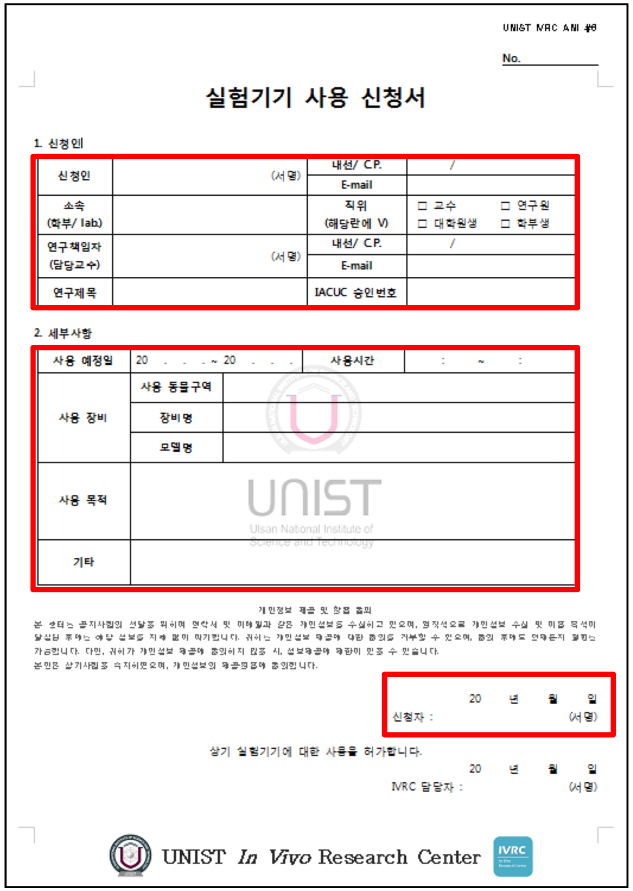
IVRC self-user system

1. UCRF 홈페이지 접속
   - UCRF homepage : http://ucrf-eng.unist.ac.kr/main/main.php
   - 자료마당 → 자료실 → 실험기기 사용 신청서 다운로드

IVRC self-user system

2. 실험기기 사용 신청서 작성 및 담당자와 조율

- 신청서 작성
- 장비이용에 관한 세부사항 (동물이동, 장비이용시간, 실험관련) 은 담당자와 조율할것



UNIST IVRC ANI #6

No. ______

실험기기 사용 신청서

1. 신청인

신청인	(서 명)	내선/ C.P.	/
		E-mail	
소속 (학부/ lab.)		직위 (해당란에 V)	□ 교수 □ 연구원 □ 대학원생 □ 학부생
연구책임자 (담당교수)	(서 명)	내선/ C.P.	/
		E-mail	
연구제목		IACUC 승인번호	

2. 세부사항

사용 예정일	20 . . . ~ 20 . . .	사용시간	: ~ :
사용 장비	사용 동물구역		
	장비명		
	모델명		
사용 목적			
기타			

개인정보 제공 및 활용 동의

본 센터는 공지사항의 전달을 위하여 연락처 및 이메일과 같은 개인정보를 수집하고 있으며, 명시적으로 개인정보 수집 및 이용 목적이 달성된 후에는 해당 정보를 지체 없이 파기합니다. 귀하는 개인정보 제공에 대한 동의를 거부할 수 있으며, 동의 후에도 언제든지 철회는 가능합니다. 다만, 귀하가 개인정보 제공에 동의하지 않을 시, 정보제공에 제한이 있을 수 있습니다.
본인은 상기사항을 숙지하였으며, 개인정보의 제공활용에 동의합니다.

20 년 월 일
신청자 : (서 명)

상기 실험기기에 대한 사용을 허가합니다.

20 년 월 일
IVRC 담당자 : (서 명)

UNIST *In Vivo* Research Center

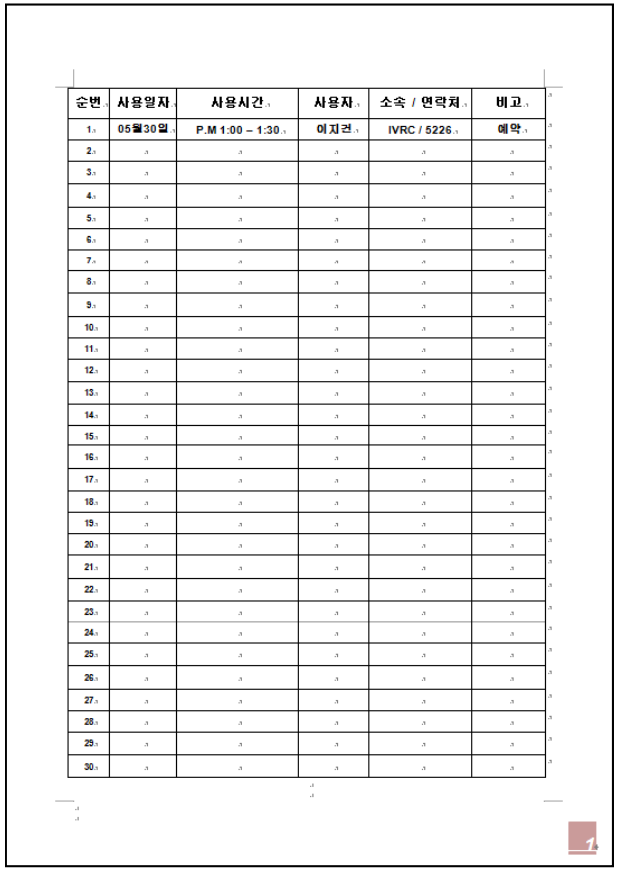
- 장비사용자의 정보를 기재

- 장비사용 예정일 및 장비사용 예정시간
- 동물구역, 실험에 필요한 장비, 사용목적을 자세하게 기재

- 서명

IVRC self-user system

3. Log book 작성 및 장비이용
   - Log book 작성
   - 정확한 장비이용 방법을 습득한 후 장비이용
   - 장비 옆에 비치되어 있음



순번	사용일자	사용시간	사용자	소속 / 연락처	비고
1	05월30일	P.M 1:00 – 1:30	이지권	IVRC / 5226	예약
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- 장비이용을 할때 반드시 log book 을 작성할것
- 사용일자, 사용시간, 사용자, 소속 및 연락처, 비고

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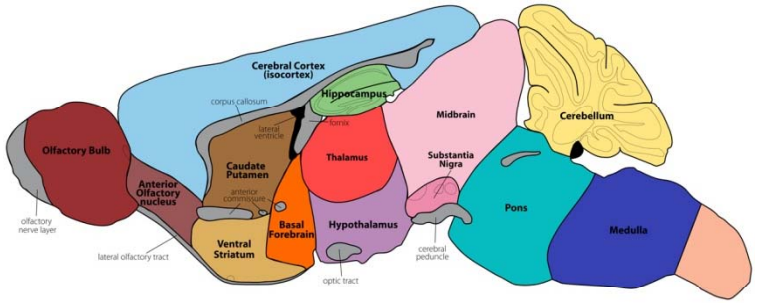
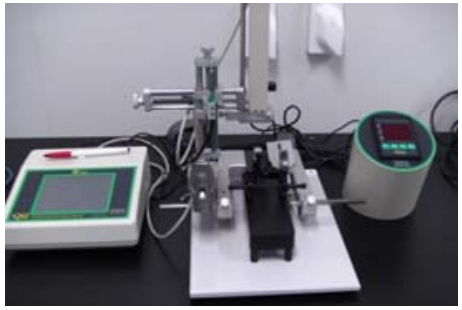
Introduction

▶ Brain research using stereotaxic surgery system

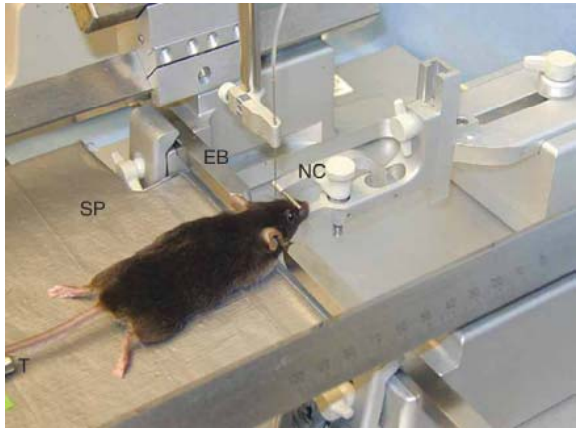
- Attractive approach for studying genetic, cellular and circuit functions in the brain
- Delivery of drug, virus vector, cell into the brain
- Cell destruction



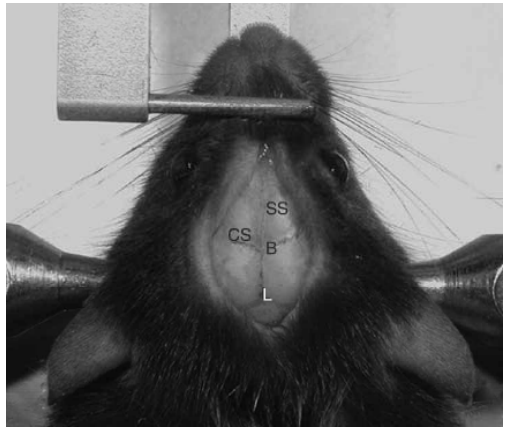
Anatomy of mouse brain



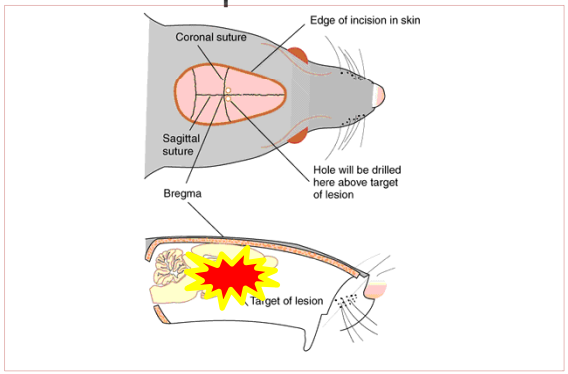
Stereotaxic surgery system



Setting



Fixation



Targeting injection

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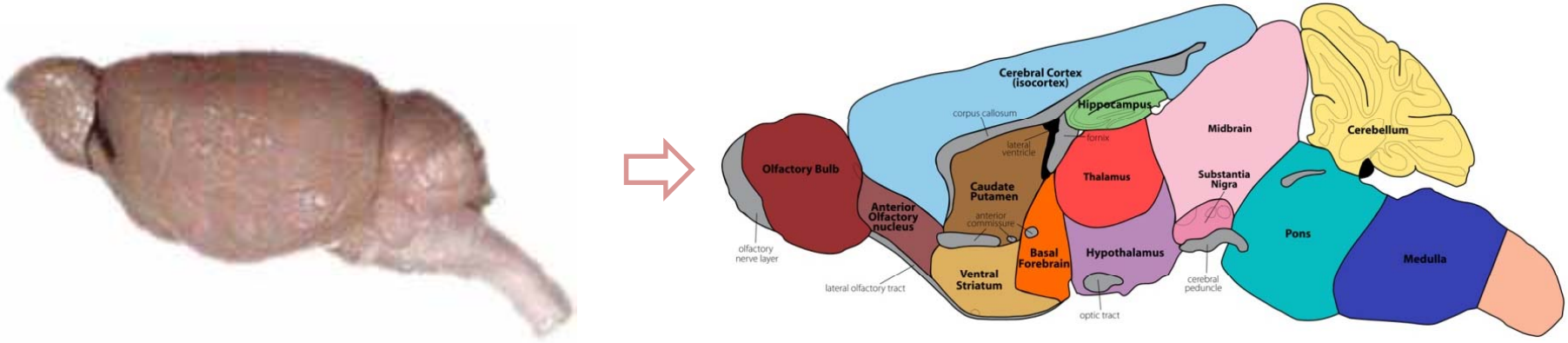
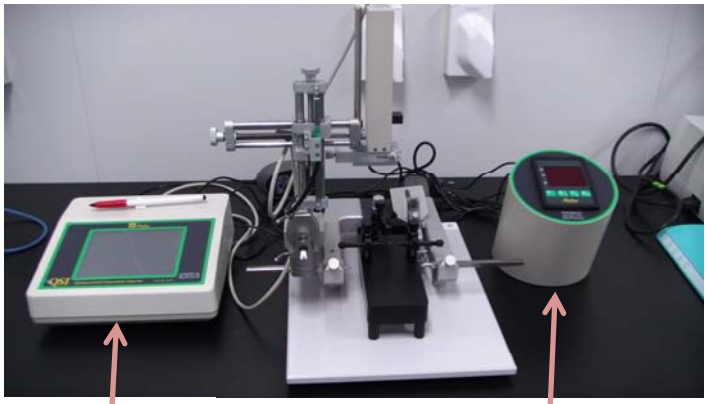
Problem of brain research



- Brain surgery poses a special problem in that usually the target cannot be located visually.
- An alternate method of locating brain structures is required.
- A method was devised whereby brain structures are located by knowing their spatial relationships to landmarks which are visible.

Stereotaxic apparatus



Electrode manipulator
- AP, ML, DV drives

DV

ML

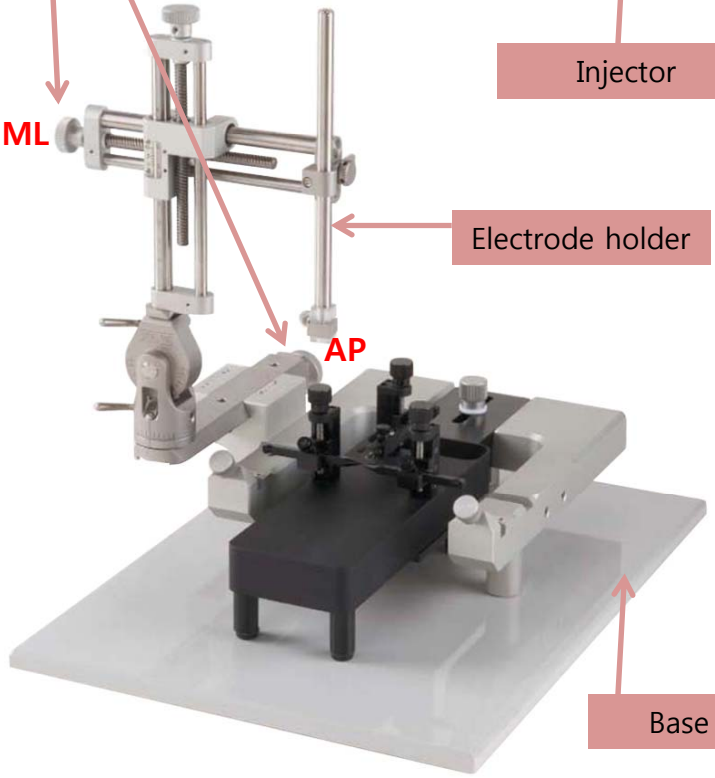
AP

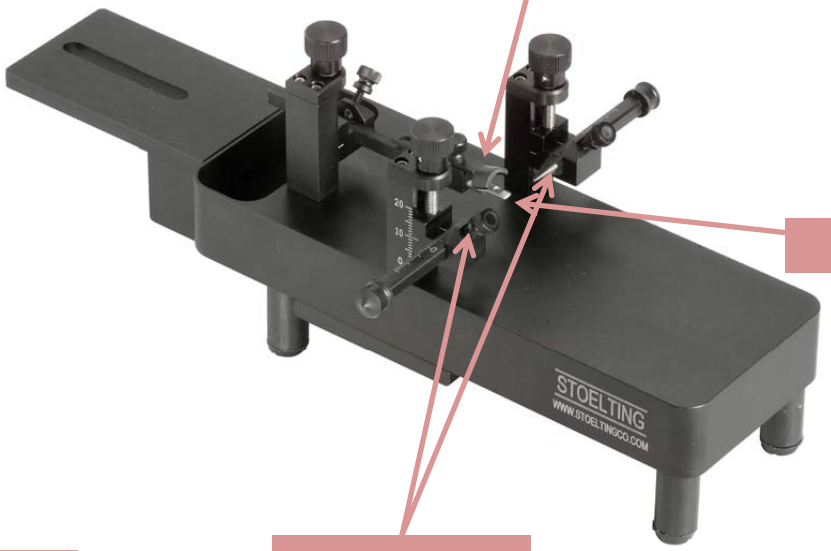
Electrode holder

Injector

LED display

Nose clamp





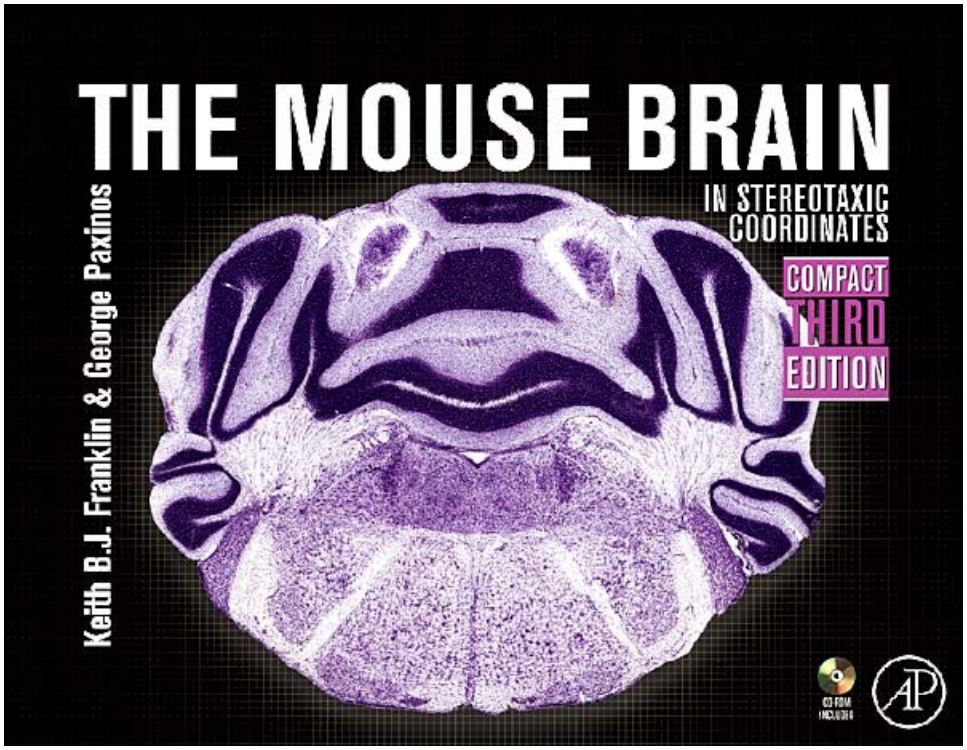
Incisor bar

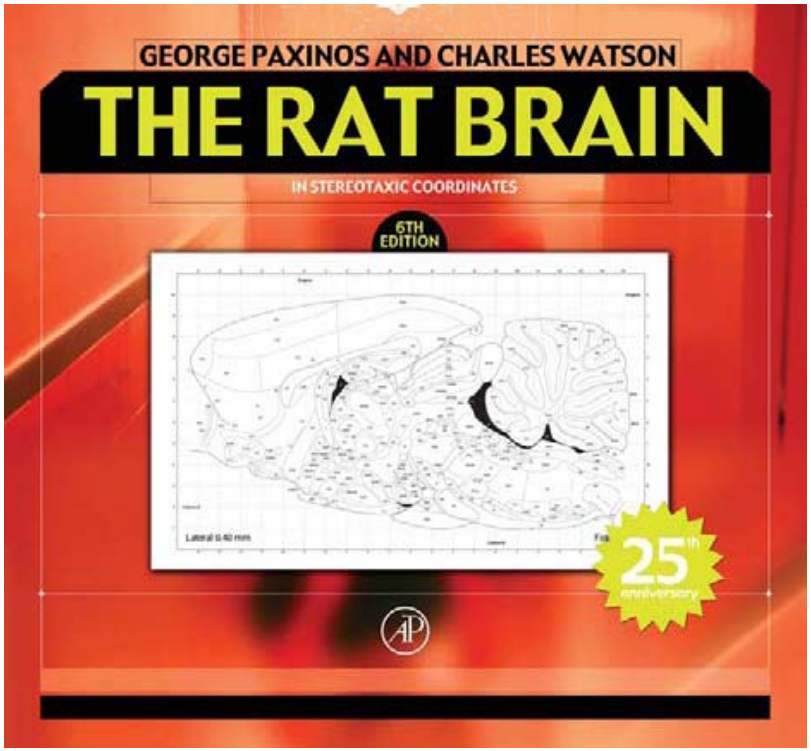
Base plate

Ear bar

Stereotaxic atlas

- Stereotaxic coordinates are usually obtained from a stereotaxic atlas.
- A stereotaxic atlas is a 3D reconstruction of the brain compiled from serial sections and drawings of sectioned brains.
- Three-dimensional (x, y and z) distances (in mm) from bregma
  : the x plane : medial-to-lateral (left-to-right) distance from bregma
  the y plane : anterior-to-posterior (front-to-back) distance from bregma
  the z plane : dorsal-to-ventral (up-and-down) distance from bregma





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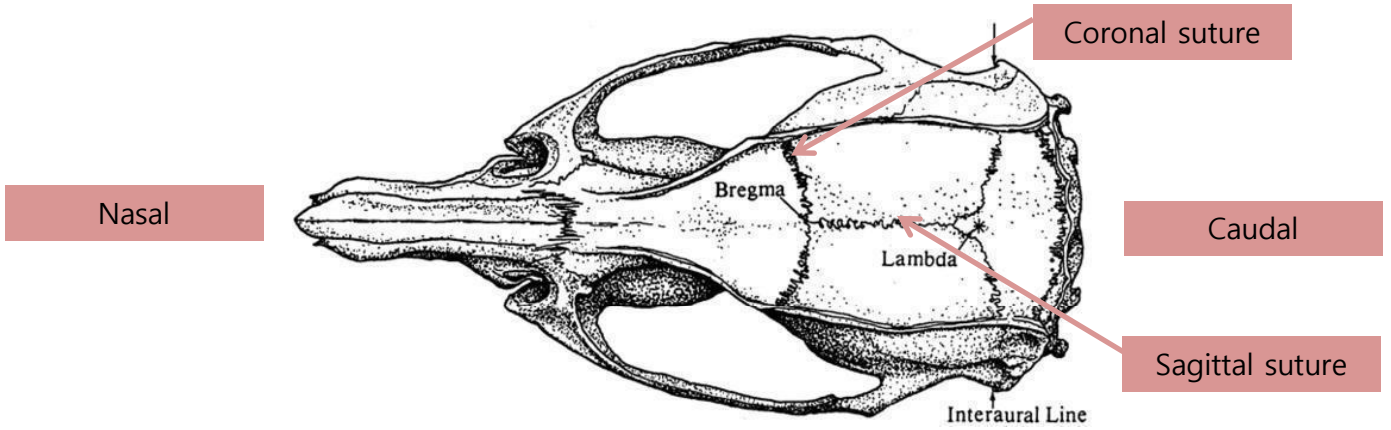
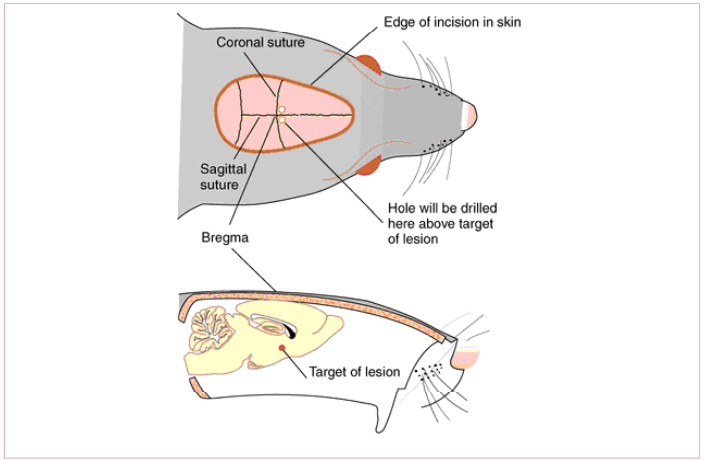
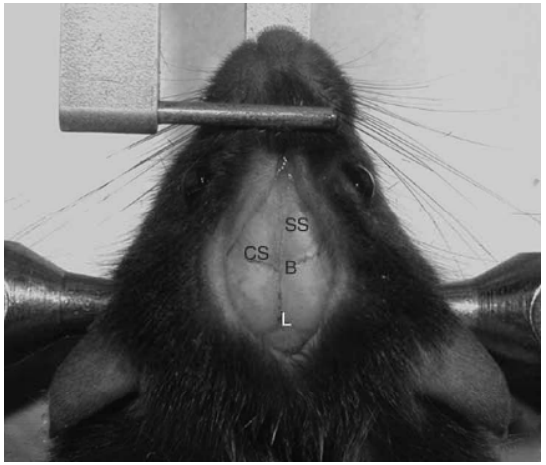
Principle of stereotaxic surgery

- The stereotaxic coordinate system does not have a single universally accepted reference point. The 3 most common reference points are bregma, lambda, and the stereotaxic zero.
- Bregma is a landmark on the skull surface where the coronal suture meets the sagittal suture.







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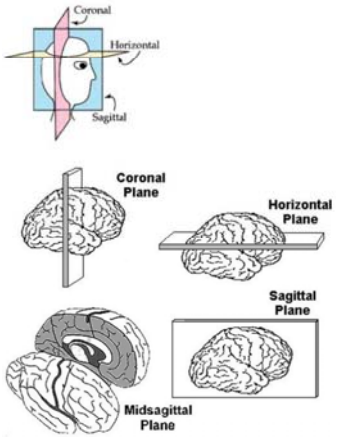
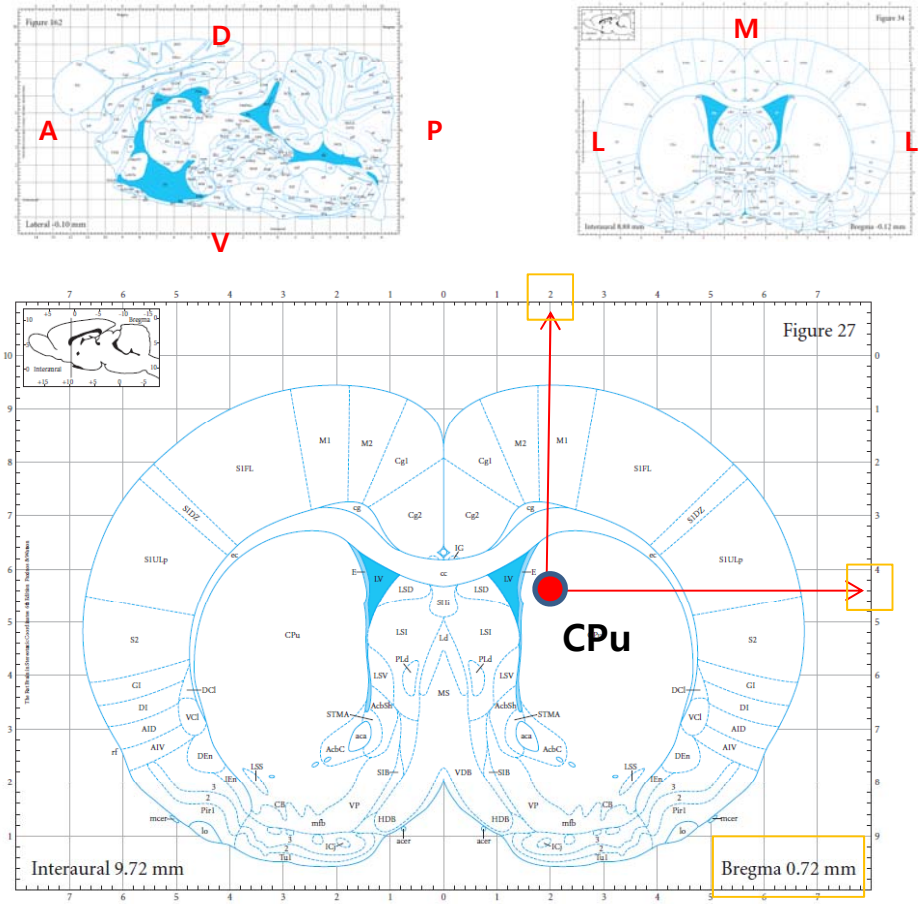
Principle of stereotaxic surgery

- To construct an atlas, brains from a particular size, sex and strain of animal are oriented to a specific orientation.
- The brain is serial sectioned.
- The spatial relationship is expressed using a set of 3 coordinates: AP, ML, DV.
  (AP : anterior-posterior, ML : medial-lateral, DV : dorsal-ventral)





Plane	Medial CPu
AP	0.72 mm
ML	2.0 mm
DV	4.4 mm

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Principle of stereotaxic surgery

Subparafascicular nucleus, parvicellular part	SPFp	C57BL/6J	174583187	-3.28	2.00	3.20	0
Subparafascicular nucleus, parvicellular part	SPFp	Th-Cre_FI172-6201	266501422	-2.92	1.75	3.45	0
Substantia innominata	SI	C57BL/6J	125436508	1.10	1.50	5.15	0
Substantia innominata	SI	Chat-IRES-Cre	126711445	-0.58	1.75	4.50	0
Substantia innominata	SI	Chat-IRES-Cre	126841788	0.02	1.60	4.55	0
Substantia innominata	SI	378-1905	273026584	0.62	1.20	4.90	0
Substantia innominata	SI	Crh-IRES-Cre (BL)-334	278179088	-0.94	2.25	4.47	0
Substantia nigra, compact part	SNc	Slc6a3-Cre	160539283	-3.08	1.25	4.08	0
Substantia nigra, reticular part	SNr	C57BL/6J	100141993	-3.28	1.50	4.30	0
Substantia nigra, reticular part	SNr	C57BL/6J	158914182	-3.08	1.25	4.30	0
Substantia nigra, reticular part	SNr	C57BL/6J	175263063	-3.40	1.60	4.60	0
Subthalamic nucleus	STN	C57BL/6J	146986331	-1.34	1.75	4.50	0
Subthalamic nucleus	STN	C57BL/6J	174788109	-1.82	2.05	4.25	0
Subthalamic nucleus	STN	A930038C07Rik-Tg1-Cre-3886	264697714	-1.70	1.40	4.85	0
Superior central nucleus raphe	CS	C57BL/6J	156929391	-4.36	0.30	4.50	0
Superior colliculus, motor related	SCm	C57BL/6J	126523066	-4.48	0.50	1.85	0
Superior colliculus, motor related	SCm	C57BL/6J	126646502	-3.80	0.30	1.20	0
Superior colliculus, motor related	SCm	C57BL/6J	128001349	-4.36	1.25	1.20	0
Superior colliculus, motor related	SCm	C57BL/6J	146078721	-3.80	1.50	1.77	0
Superior colliculus, motor related	SCm	Pdzk1ip1-Cre_KD31	167118084	-2.92	1.25	2.76	0
Superior colliculus, motor related	SCm	C57BL/6J	175158132	-3.80	1.50	2.10	0

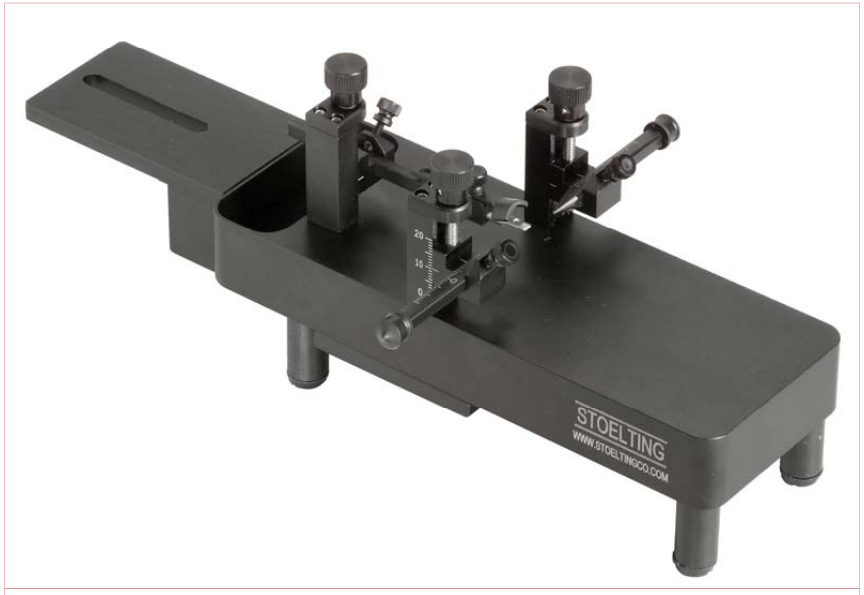
Procedure of stereotaxic surgery

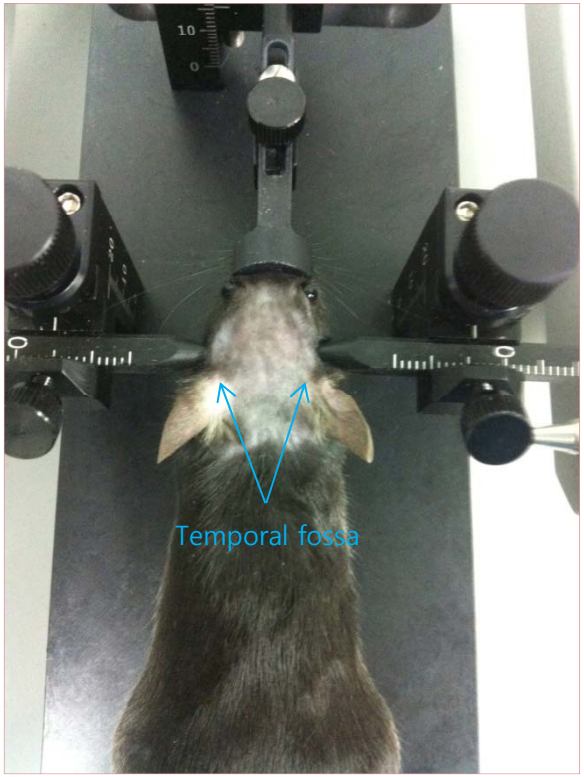
1. Anesthesia of animal
   - ▶ Injection
   - ▶ Volatile anesthesia

     ex) isoflurane

Procedure of stereotaxic surgery

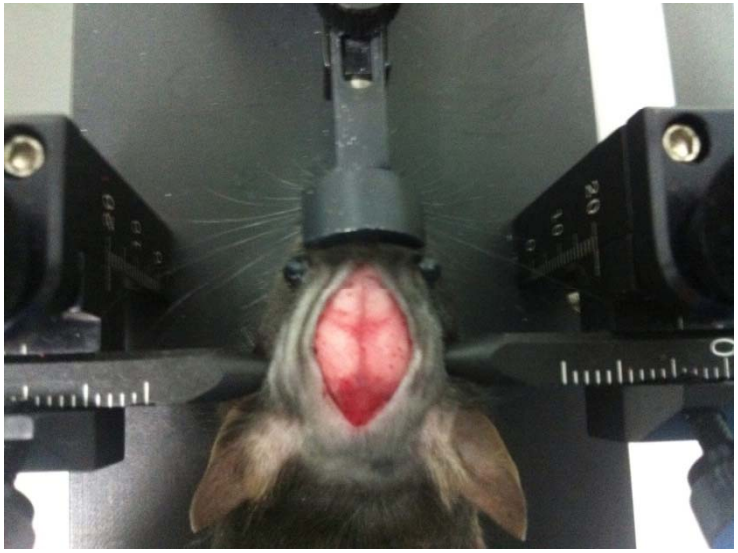
2. Fixation of mouse head
   - ▶ Incisor bar
   - ▶ Nose clamp
   - ▶ Ear bar

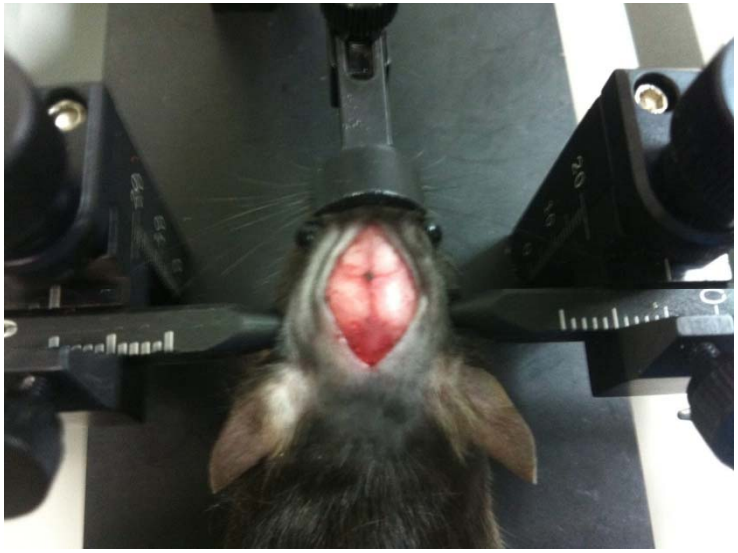




Procedure of stereotaxic surgery

3. Marking of bregma
   - ▶ Incision of head skin
   - ▶ Using bone scraper or 면봉
   - ▶ Marking bregma





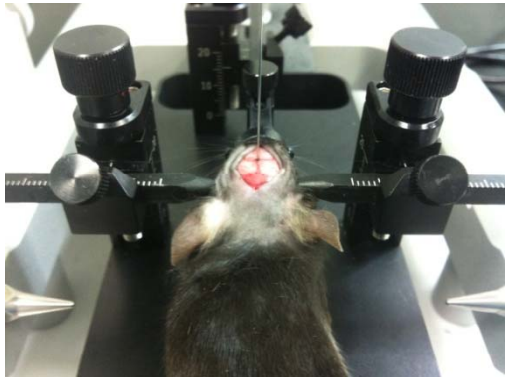
Procedure of stereotaxic surgery

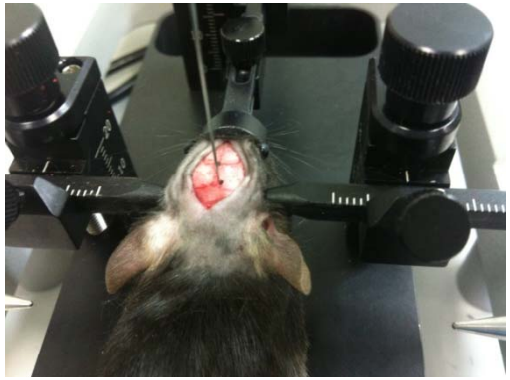
4. Marking of targeting region

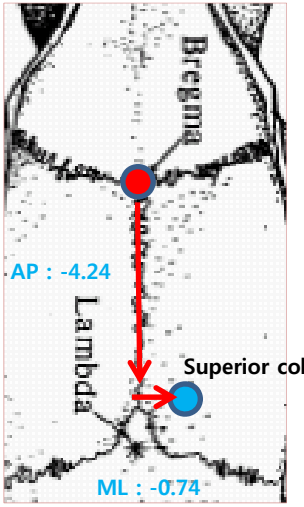
▶ Checking the targeting region using atlas

(ex : superior colliculus; AP = -4.24, ML = -0.75, DV = -1)

▶ Marking target region using sylinge carrier

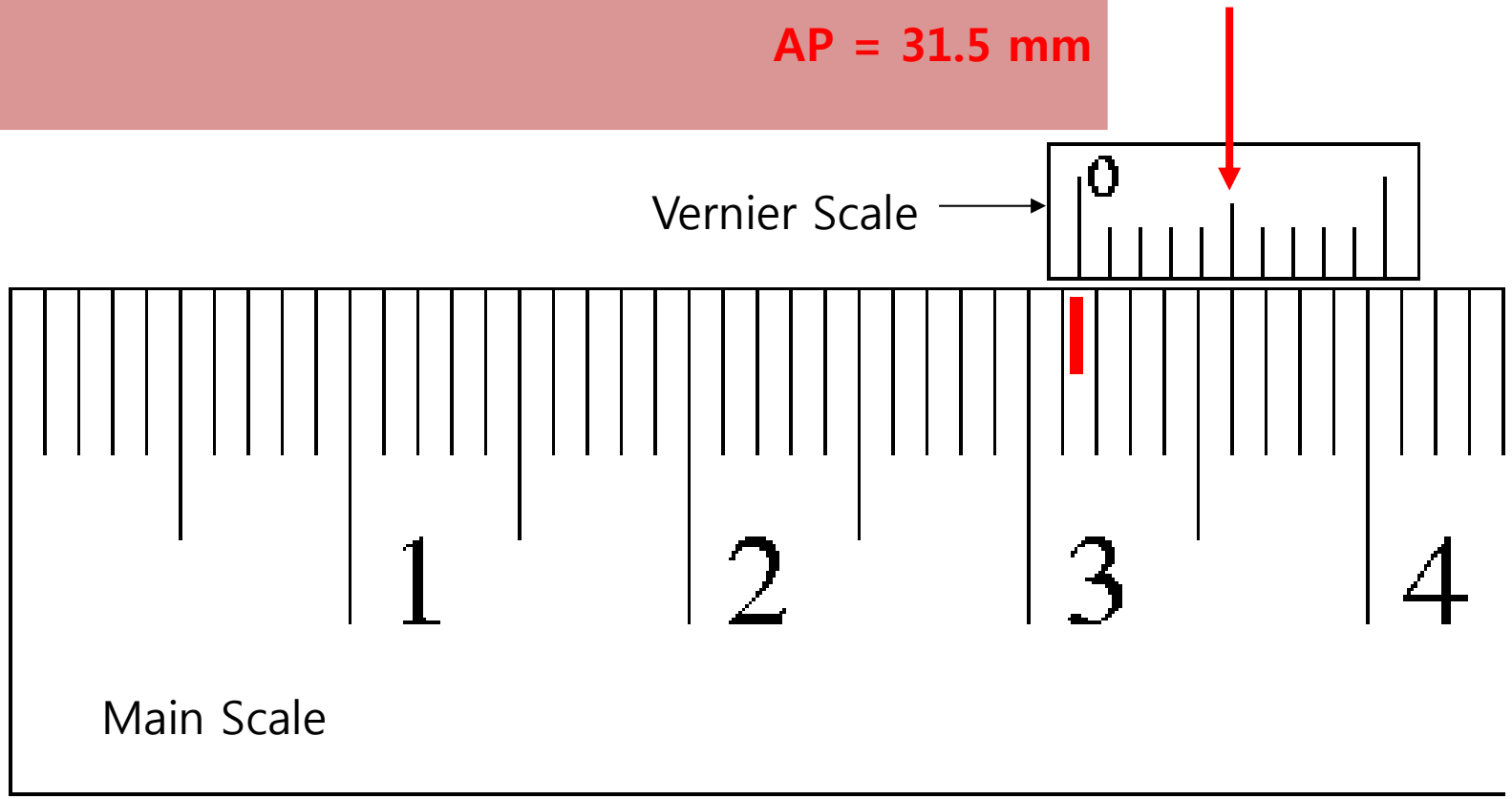






The "0" line on the Vernier is above 3.1 cm (or 31 mm) on the main scale. To determine the 10th of a mm, estimate which line on the Vernier scale lines up best with a line on the main scale.

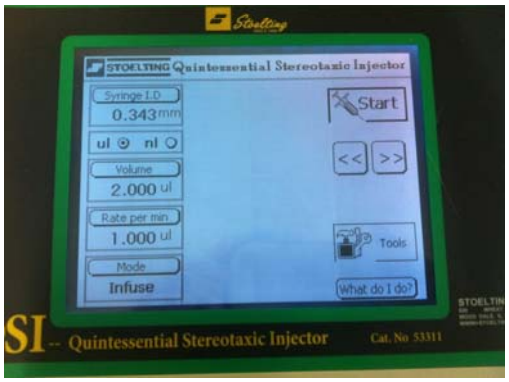
AP = 31.5 mm



Procedure of stereotaxic surgery

5. Drilling skull & injection

- ▶ Micro-drilling of skull in the targeting region
- ▶ Injection using sylinge carrier (DV = -1 from pia mater)



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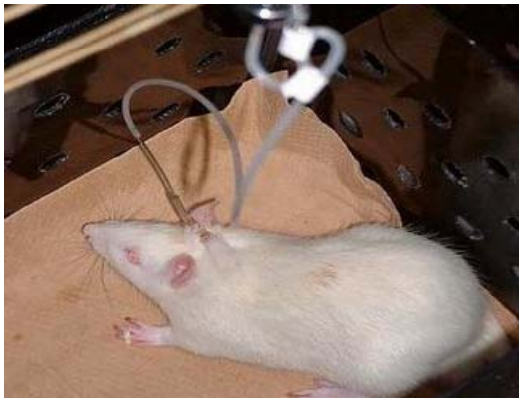
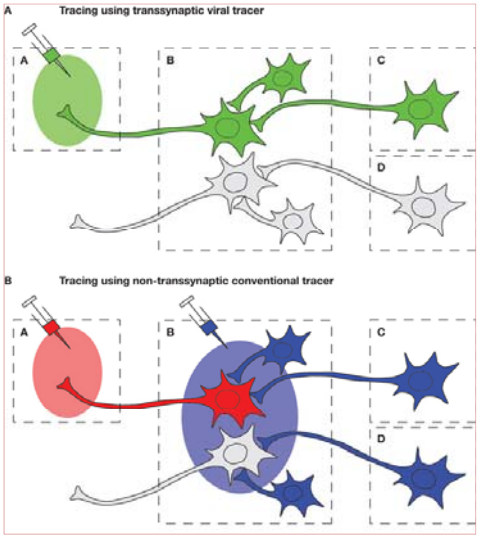
Application of stereotaxic surgery

Strereotaxic system has many techniques including.

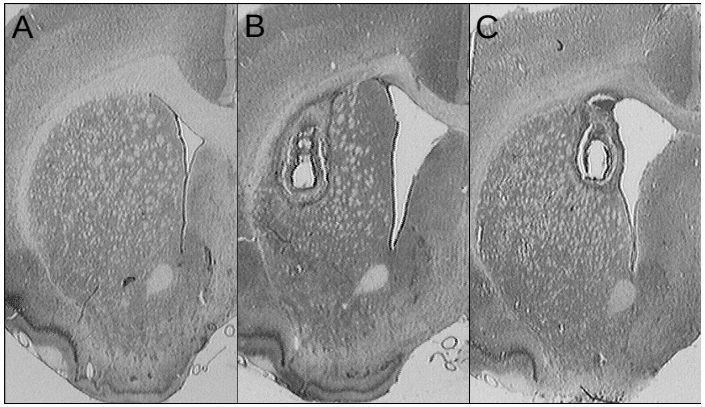
- Drug delivery
- Tracing neural connection
- Lesion methods



Intracerebral (i.c.) injection



Tracing neural connection



Electrolytic lesions of the CPu