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Tissue Punching Protocol

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ABSTRACT

SOP for brain punching for the Biobanks

ATTACHMENTS

PunchingAtlas1.ai

PROTOCOL CITATION

McKenzie Pavlich, Lani Tieu, Brent Boomhower, Lisa Maturin, Giordano De Guglielmo, Olivier George 2021. Tissue Punching Protocol. **protocols.io** https://protocols.io/view/tissue-punching-protocol-brgqm3vw

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46320

Beginning Prep

1 Take brain out of -80° freezer and leave in -20 ° freezer overnight.

2 Make sure the cryostat is at -14° and the stage matches the cryostat temperature. If you plan on leaving the lid to the cryostat open during punching, you may want to turn the temperature a few degrees colder as the temperature warms up with the lid open.

3 Turn on cryostat light.

4 Get dry ice for tubes and crush it to fill a tube holder.

5 Label tubes for all desired regions.

6 ⚠

Make sure that the cryostat blade is sharp (must be sharpened at least every 6-12 months)

7 Place blade in cryostat and make sure there is a good blade/plate ratio.

8 Find punching tools and place them nearby in crushed dry ice.

Punching

9 When you are ready to begin, move brain from -20° freezer to cryostat. Let sit for ~5 min.

10 Cut off 1/3 of cerebellum to create a flat surface for the brain to stand on.

11 Add layers of OCT compound glue until the glue is over the weak joint point where the cerebellum connects. When adding additional layers of glue, make sure that the previous layer has frozen completely before adding the next layer.

12 Once the glue has been added and is completely frozen place chuck on the stage and adjust the brain to correct for any slanting that occurred while cutting the cerebellum.

13 Begin cutting the brain at 50-100um thickness until you get to region of interest. It is OK to adjust stage during this process to make sure you are getting a full slice of the brain.

14 When you start to see your region of interest, switch the cutting thickness to 500um.

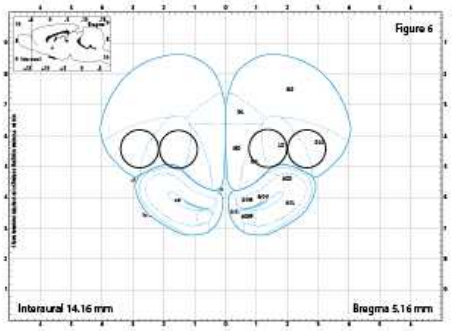
15 Use the punching tools to make a mark where you would like to punch. Please see attached images for regions collected.

15.1

Each section represents the first section of a stack
Stack thickness = number of 50 µm sections
(20=1000 µm) ▼

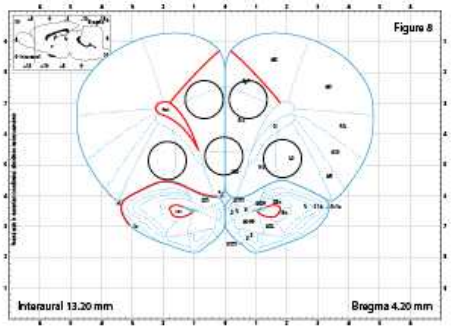
PFC 16g
▼
size of punch

Tube Number



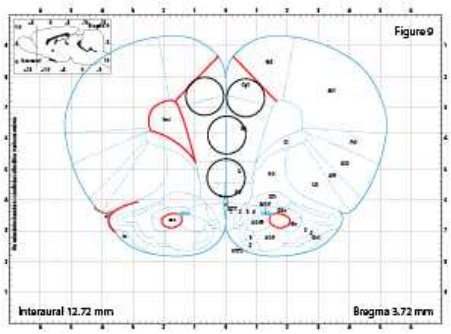
20 **Image 1**

OFC 16g (1)



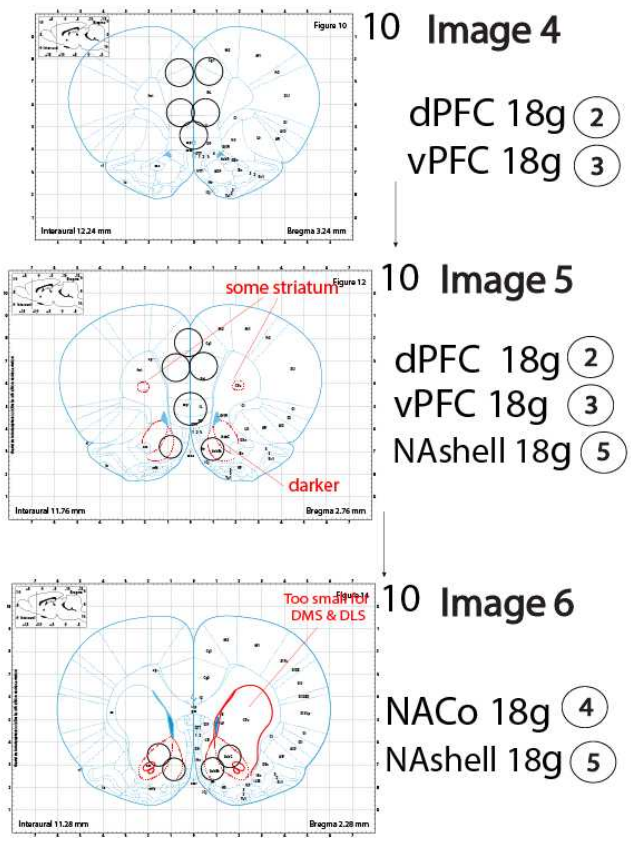
10 **Image 2**

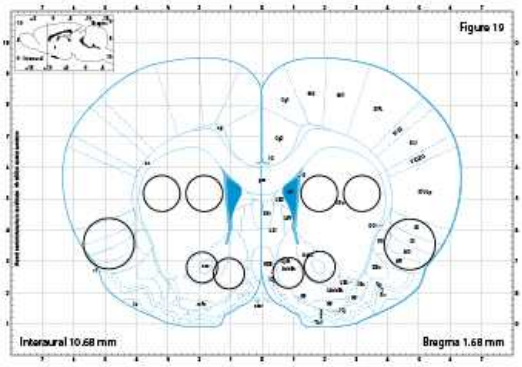
dPFC 18g (2)
vPFC 18g (3)
OFC 18g (1)



10 **Image 3**

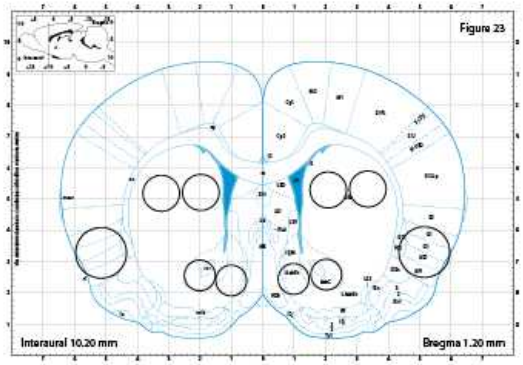
dPFC 18g (2)
vPFC 18g (3)





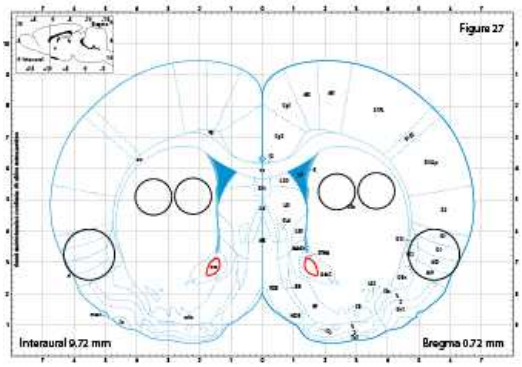
10 **Image 7**

DLS 18g 7
DMS 18g 6
Insula 18g 8
NACo 18g 4
NAshell 18g 5



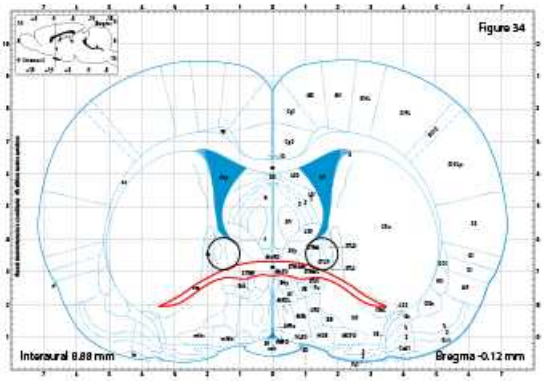
10 **Image 8**

DLS 18g 7
DMS 18g 6
Insula 18g 8
NACo 18g 4
NAshell 18g 5



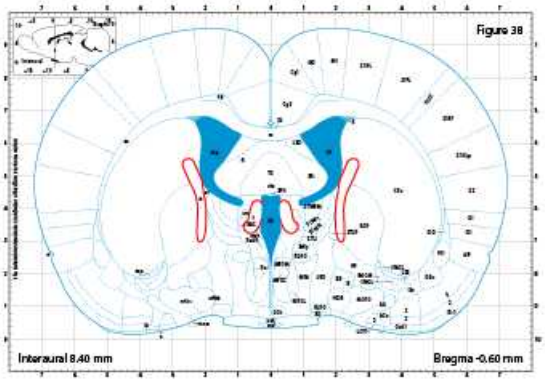
10 **Image 9**

DLS 18g 7
DMS 18g 6
Insula 18g 8



10 Image 10

Bnst 18g 9



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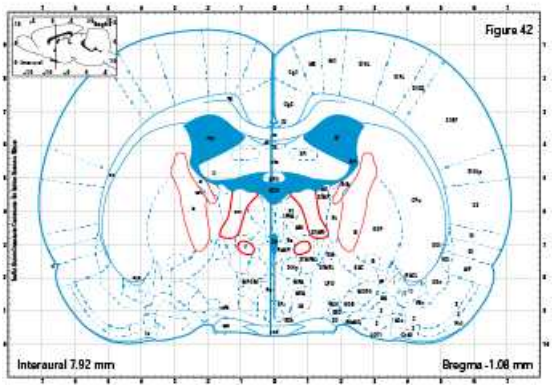

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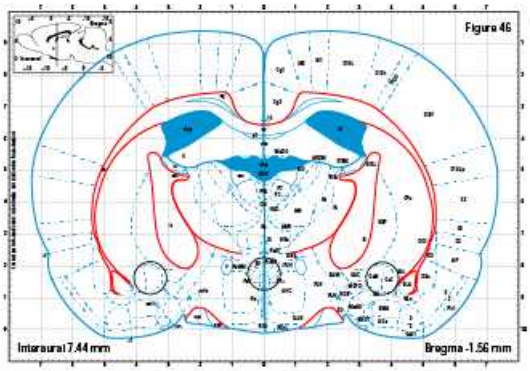
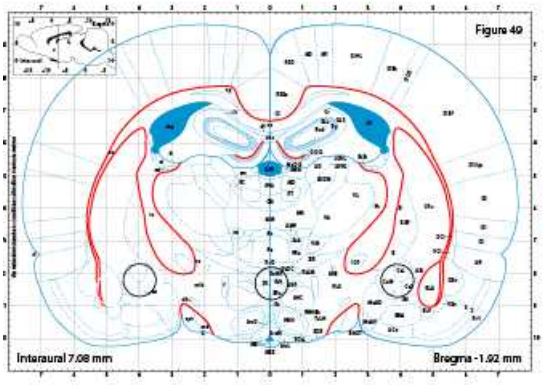


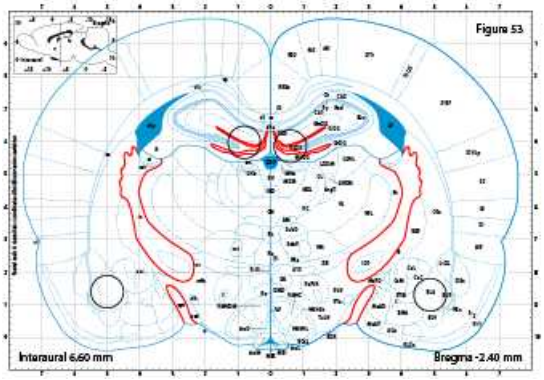
10 Image 11

CeA 18g (10)
PVN 18g (12)



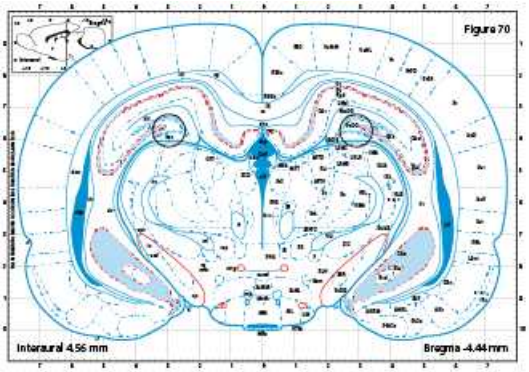
10 Image 12

CeA 18g (10)
PVN 18g (12)



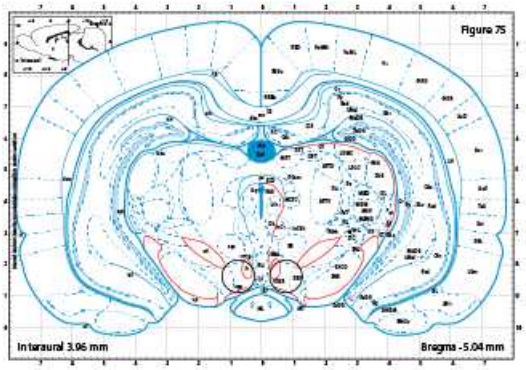
10 Image 13

BLA 18g (11)
DG 18g (13)



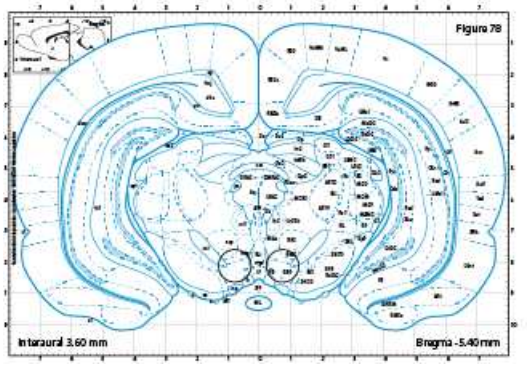
10 Image 14

DG 18g (13)



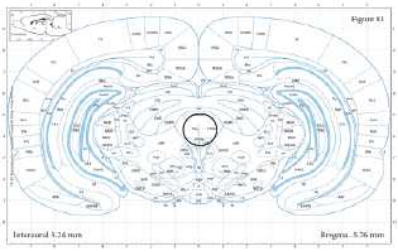
10 Image 15

VTA 18g (14)



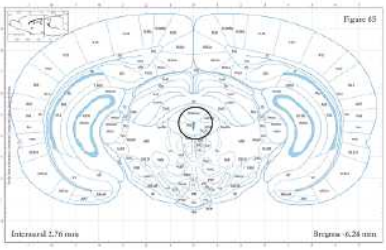
10 Image 16

VTA 18g (14)



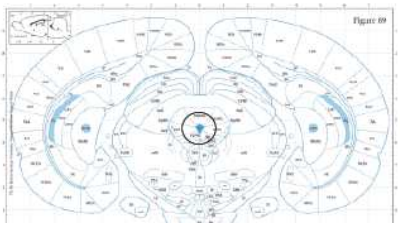
10 Image 17

PAG 16g (15)



10 Image 18

PAG 16g (15)



10 Image 19

PAG 16g (15)

16 Cut the brain slice at 500um and take punches that correspond with your markings.

17 Place punching tool in tube that has a label that matches the punched region. Leave in tube for ~10-15 seconds and then punch the tissue out with punching rod. If you do not see the pellet drop, it could have melted to the side of the tool. Ensure the punch is frozen and collected at the bottom of the tube before moving on.

18 ⚠

ALWAYS PLACE THE PUNCHING TOOLS BACK IN DRY ICE BETWEEN PUNCHES

19 Make tally marks as you complete punches for each region.

20 When you are finished with punching, place all tubes with tissue in the -80° freezer

Clean Up

21 Remove blade.

22 Clear away discarded tissue sections.

23 Clean all chucks used.

24 Turn off cryostat light and set cryostat back to desired temperature.

25 Return the stage to the home position.

26 Put all tools away.

27 Clean surrounding area.