

Index

Note: Page numbers followed by f, t, or b denote a figure, table, or box respectively on the corresponding page.

- A**
- Abrupt, 70
 - Acetylcholine, 157–158, 851
 - Acetylcholine receptors (AChRs), 157–158
 - Acoustic communication during reproductive behaviors (protocol), 730–735, 731f
 - audio playback, 733
 - audio recording, 733
 - calibration of playback setup, 732
 - data analysis, 733–734
 - discussion, 735
 - fly collection and transfer, 732–733
 - materials, 730–731, 731f
 - method, 732–734
 - troubleshooting, 734–735
 - ActiMetrics, 866
 - Actin blob, 71
 - Actin patch, 71–72
 - Action potentials
 - extracellular recording, 343–345, 344t
 - intracellular recording, 344t, 345
 - phases, 357
 - Actogram], 866
 - AD/DA interface board, 364
 - Adipokinetic hormone, 660
 - Adult external saline (recipe), 452, 464–465
 - Adult internal saline (recipe), 440, 465
 - Adults
 - classical conditioning, 811–844
 - in *Drosophila* life cycle, 3
 - mitochondrial dynamics in adult axons, analysis of, 633–653
 - nociception in, 766
 - Aequorin, 614
 - Agar (1.5%)–noni juice (50%) (recipe), 894
 - Agarose channels
 - design, 223
 - generating linear, 222–223
 - imaging of larvae in, 217–219, 218f
 - mold manufacturing, 223–224
 - production of negative molds, 216
 - production of positive molds, 223–224
 - studying larval behavioral (protocol), 214–226, 218f
 - Agar plates (1%, w/v) (recipe), 785
 - Agar (4%) plates (recipe), 101
 - Agar (1.5%)–sucrose (2%) (recipe), 895
 - Aggression, 845–862
 - behavioral patterns, 846–848, 846t, 847f
 - external sensory cues, 848–849
 - intensity of encounters, 850
 - methods for quantifying and analyzing aggression (protocol), 855–862
 - overview, 845–846
 - Aggression, methods for quantifying and analyzing (protocol), 855–862
 - discussion, 861–862
 - divider chamber assay, 858f, 858t, 859–861
 - materials, 855–857
 - method, 857–861, 858f, 858t
 - multiwell chamber assay, 857, 858f, 858t, 859
 - pupae isolation, 857
 - recipe, 862
 - scoring and quantifying aggression, 861
 - sliding chamber assay, 858f, 858t, 859
 - Alcohol dehydrogenase, 746
 - Alexa Fluor 568 stock solution (5 mM), 426
 - All-*trans*-retinal (ATR) (recipe), 779
 - All-*trans*-retinal fly food (recipe), 807
 - All-*trans*-retinal (ATR) food medium, 755–756, 871
 - All-*trans*-retinal stock solution (100 mM) (recipe), 888
 - All-*trans*-retinal stock solution (200 mM) (recipe), 807
 - amnesiac*, 747
 - Amplifier, 363–364
 - Anesthesia-resistant memory (ARM), 812
 - Antennal and brain samples
 - chromatin immunoprecipitation (ChIP) (protocol), 310–316
 - qPCR after ChIP of samples, 300–309, 304f
 - sample preparation for chromatin immunoprecipitation (ChIP) (protocol), 300–309, 304f
 - Antennal sensilla
 - olfactory, 532, 533f
 - recording from, 552–553
 - Antibodies, for labeling neural subsets in *Drosophila*, 9t
 - Aphrodisiac cues, 706–707
 - Appetitive conditioning
 - background, 812–813
 - classical conditioning (protocol), 816–842
 - olfactory, 832–836
 - Appetitive odor–taste learning, 674
 - Appetitive olfactory conditioning
 - procedure, 832–836
 - agar vial preparation, 832
 - appetitive training, 834–835
 - dilution of odors, 833
 - food deprivation, 833, 834f
 - performance index, calculating, 836
 - reciprocal appetitive training, 836
 - sucrose and blank papers, preparing, 832–833, 833f
 - testing, 835
 - T-maze apparatus setup for, 833, 834f
 - ArcLight, 346, 588
 - Arp2/3, 72
 - ASAP2, 346
 - asense*, 636
 - Associative learning, olfactory, 583–584
 - Associative memory
 - assay for olfactory, 671–675
 - taste memory, 692–693
 - Aversive conditioning
 - background, 812
 - classical conditioning (protocol), 816–842
 - olfactory, 828–832
 - varying test odor choice and context, 812
 - Aversive odor–high salt learning, 672–673
 - Aversive odor–taste learning, 673–674, 678–689
 - Aversive olfactory conditioning procedure, 828–832
 - aversive training, 830–831
 - dilution of odors, 829
 - electric shock stimulator settings, 830
 - memory performance, calculating, 832
 - preparation of flies, 832–833, 832f
 - reciprocal aversive training, 831
 - testing, 831
 - T-maze setup, 829–830, 829f
 - Aversive olfactory–taste learning and memory in larvae (protocol), 678–689, 680f–681f, 683f–684f
 - assay plate preparation, 680, 681f–682f
 - data analysis, 684–685, 684f
 - discussion, 686–688, 687f

Index

- Aversive olfactory–taste learning and memory in larvae (protocol) (*Continued*)
 larvae preparation, 681–682
 materials, 678–679
 method, 679–685, 680f–681f, 683f–684f
 odor container preparation, 681
 rearing larvae, 679
 reciprocal training, 683–684
 testing, 682, 683f
 training, 682
- Aversive taste memory, 692–693, 703
- Avoidance, dendritic, 72–73
- Axoclamp-2B, 365
- Axonal injury and degeneration in *Drosophila*, 31–66
 immobilizing second-instar *Drosophila* larvae for imaging and surgery using the larva chip (protocol), 51–57, 53f–54f
 introduction, 31–33
 laser microsurgery in *Drosophila* larvae using the MicroPoint ablation system (protocol), 58–66, 60f–62f
 peripheral nerve crush in *Drosophila* larvae (protocol), 41–50, 43f, 45f
 Wallerian degeneration and clearance of olfactory receptor neuron axons following *Drosophila* antennal transection (protocol), 35–40, 37f
- Axon guidance, 5–30
 at CNS midline, 6–7, 7f–8f
 collection, fixation, and antibody staining of *Drosophila* embryos (protocol), 15–24
 commissureless epistasis, 7–9
 immunohistochemistry *versus* immunofluorescence, 10–12
 introduction, 5–6
 longitudinal, 9–10
 in optic lobe, 26f
 ventral nerve cord dissection and microscopy of *Drosophila* embryos (protocol), 25–30
- Axons
 peripheral nerve crush in *Drosophila* larvae (protocol), 41–50, 43f, 45f
 Wallerian degeneration and clearance of olfactory receptor neuron axons following *Drosophila* antennal transection (protocol), 35–40, 37f
- Axundead (Axed)*, 32
- B**
- Baines solution, 244, 258
- Balancer chromosome, 15, 229, 335, 336f, 337, 666
- Bantam, 74
barfly, 747
- Basigin, 127
- Beadle–Ephrussi ringer solution (recipe), 567
- Beam breaks for sleep measurement, 864–867
 hardware, 865–866
 introduction, 864–865
 software, 866–867
 video-based tracking compared, 867–868
- Behavior
 aggression, 845–862
 classical conditioning, 811–844
 energy homeostasis, 659–670
 ethanol responses, 745–762
 introduction to studying, 655–657
 learning and memory in larvae, 671–689
 nociception, 763–787
 reproductive, 705–744, 706f
 sleep, 863–905
 studying larval in agarose channels (protocol), 214–226, 218f
 taste processing, 691–704
 tracking navigation, 789–809, 790f
- Bernstein, Julius, 350
- Biological safety procedures, 911
- Birthdating, of neural stem cell lineages, 284, 287–294, 288f
- Blocking solution for *Drosophila* staining (recipe), 23
- Blocking solution II for *Drosophila* larvae (recipe), 154
- Blood–brain barrier, 124–125
- Body fat determination, density assay for, 661, 663–670, 669f
- Body wall dissection tools and techniques (protocol), 168–178, 171f, 173f, 175f
 embryo collection, 172
 late-embryo dissection technique, 172–174, 173f
 materials, 168–169
 method, 170–176, 171f
 recipes, 176–177
 sharpened tungsten tools, generating ideal, 170–172, 171f
 standard larval dissection and fixation for immunolabeling, 174–176, 175f
- Body wall NMJ preparations, immunohistochemistry and morphometric analysis of larval (protocol), 179–185, 181f–182f
 antibody signal enhancement, 182–183, 182f
 immunolabeling of tissue, 180–181
 materials, 179–180
 method, 180–184, 181f–182f
 morphometric analysis (scoring bouton number and growth), 183–184
 recipes, 184
 signal amplification using tertiary antibodies, 182–183, 182f
- Booz-o-mat, 747
- BORIS, 716, 718
- Boutons
 development of, 159
 focal recording of synaptic currents from single boutons at larval NMJ (protocol), 390–393
 identification, 181f
 number, 163, 183–184
 types, 160–161
- Brain
 explant preparation, 607–608
 ex vivo imaging (protocol), 605–611
 in vivo imaging of olfactory learning-induced plasticity in (protocol), 594–604, 596f
- Brain dissection
 adult, 273–274, 274f
 larval, 272
 for patch-clamping neurons, 448–454, 449f
 pupal, 272–273, 273f
- Brain–fat body axis, 659
- Brain samples
 chromatin immunoprecipitation (ChIP) (protocol), 310–316
 preparation for chromatin immunoprecipitation (ChIP) (protocol), 300–309, 304f
 qPCR after ChIP of, 300–309, 304f
- Bridge balance, 368, 370–371, 371f, 376
- Brivido-1 (Brv1), 69
- C**
- Cacophony, 159
- Calcium
 intracellular and neuronal activity, 613–614
 signaling and neuromuscular circuit development, 159
- Calcium-dependent short-term synaptic plasticity, 379, 380f, 381
- Calcium imaging
 neuronal activity, 346, 509–530
 during sleep and wake, 467–508
 two-photon in visual neurons, 511–512

- visualization of neural activity using, 510–511
- whole-brain during sleep and wake (protocol), 489–508
- Calcium imaging of neural activity in photoreceptors, 509–530
 - background, 509–513
 - dissection of live fly head for functional imaging (protocol), 516–520, 517f–518f
 - functional imaging from photoreceptors (protocol), 521–526, 522f, 524f
 - multiplexing neural recordings, 512–513
 - presenting realistic neural activities, 513
 - quantitative analysis of photoreceptor intensity-response function in visual neurons (protocol), 527–530, 528f
 - two-photon imaging, 511–512
- Calcium-induced calcium release, 613
- Calcium influx at larval neuromuscular junction (protocol), 422–427, 425f
- Calcium loading dye (recipe), 426
- Calcium-modulated photoactivatable ratiometric integrator (CaMPARI), 614–615, 615t
- CaLexA
 - design principles, 615–617
 - example applications, 617–618
 - fly lines available, 625t
 - imaging signals in dissected fly CNS (protocol), 624–631
 - parameters to be considered when using, 617
- Calmodulin, 510, 586, 614
- Cameleon, 614
- Camera
 - digital, 571
 - scientific complementary metal oxide semiconductor (sCMOS), 571
- CaMPARI, 586
- Capacitance (C), 350b, 351b
- Capacitance compensation, 368–370, 370f, 376
- Cas9
 - about, 323–325, 324f
 - generating CRISPR alleles in *Drosophila* (protocol), 327, 329–330, 332–335, 339
- Ceiling effect, 357, 384
- Cell ablation techniques for larval neuromuscular system (protocol), 198–204
 - discussion, 202
 - genetic means of cell ablation, 199–200, 201f
 - light-induced cell ablation, 200–202, 201f
 - materials, 198–199
 - method, 199–202, 201f
 - recipes, 203
- Cell class–lineage intersection (CLIn), 287–293, 288f
- Cell surface protein labeling at larval NMJ using binding partner peptides (protocol), 186–192, 188f
 - dissection and incubation with ECD fragments, 189–190
 - ECD fragment design and production, 188–189
 - materials, 186–187
 - method, 187–190, 188f
 - recipes, 191
 - troubleshooting, 190
- Cellular circuits, electric circuits compared, 349
- Central complex
 - structure and function, 281
 - surgical procedures to expose, 497–499
- Central complex lineages
 - developmental genetic and molecular analysis of, 279–294
 - introduction, 279–281
 - larval neural stem cell markers, 281–284, 282t, 283f
 - lineage analysis and birthdating (protocol), 287–294, 288f
- Central nervous system (CNS)
 - anatomy of larval, 208–209, 208f
 - dissection and immunolabeling of larvae (protocol), 131–138, 133f
 - functional imaging of learning-induced plasticity in, 583–611
- Centrosomin, 70
- Channelrhodopsin-2 (ChR2), 775, 777–779, 791, 850
- Charge (Q), 350b
- cheapdate*, 747
- Chemical nociception, 764
- Chemicals, hazardous, 911–912
- Chemosensation
 - background, 531–532
 - molecular biology of, 534–536
- Chemosensory coding in single sensilla, 531–568
 - background, 531–532
 - chemosensory organ anatomy, 532–534, 533f
 - electrophysiological recordings from chemosensory sensilla, 536–538
 - molecular biology of chemosensation, 534–536
 - principles, 538–540
- Chemosensory electrophysiological recordings, 531–568
 - from olfactory sensilla (protocol), 545–557, 548f, 550f–551f, 554f
 - from taste sensilla (protocol), 558–568, 561f–562f, 566f
- Chemosensory organ anatomy, 532–534, 533f
- Chemotaxis
 - overview, 791
 - tracking navigation behavior in odor gradients, 791, 795–809, 802f–806f
- ChIP buffer 1 (recipe), 314
- ChIP elution buffer for *Drosophila* (recipe), 314
- ChIP lysis buffer (recipe), 307
- Chlorination of silver wires, 457b
- Chordotonal, 69, 233, 849
- ChR2 (channelrhodopsin-2), 775, 777–779, 791, 850
- Chrimson, 791–792, 801, 869–871
- Chromatin fragmentation, 297, 303, 306
- Chromatin immunoprecipitation (ChIP)
 - of antennal and brain samples (protocol), 310–316
 - cross-linked (X-ChIP), 297, 306
 - native (N-ChIP), 297
 - optimization of parameters, 298
 - overview, 295–296, 296f
 - qPCR of antennal and brain sample, 317–321
 - sample preparation of antennal and brain samples (protocol), 300–309, 304f
 - steps, 296f, 297–298
 - study of chromatin state, 295–321
 - uses in *Drosophila*, 296, 306
- Chromatin immunoprecipitation (ChIP) sample preparation (protocol), 300–309, 304f
 - discussion, 306
 - DNA extraction, 305
 - homogenization and cell lysis, 302–303
 - materials, 300–301
 - method, 301–305
 - optimization of fixation and sonication, 303–305, 304f
 - recipes, 307–308
 - sample dissection, 301–302
 - sonication, 303, 306
- Chromatin immunoprecipitation (ChIP) using antennal and brain samples (protocol), 310–316
 - materials, 310–311
 - method, 311–314
 - recipes, 314–316

Index

- Chromatin state, chromatin
 immunoprecipitation (ChIP)
 to study, 295–321
- Circadian rhythms
 activity monitoring, 863–868, 871, 874
 analysis from DAM data using SCAMP
 (protocol), 896–905
 CaLexA and, 617
 neural stimulation during DAM-based
 studies of (protocol),
 879–889
 neuropeptide release by clock neurons,
 574
 perineurial glia, 125
 positional preference analysis using
 multibeam activity monitors
 (protocol), 890–895
 timing of aggressive behavior, 849
 TRIC and, 620
- Circuit motifs, 206, 211, 509, 589, 657
- Clampex, 460–461, 464
- Clampfit, 464
- Classical conditioning
 appetitive, 812–813, 816–842
 aversive, 812, 816–842
 introduction, 811–812
- Classical conditioning of adult *Drosophila*
 (protocol), 816–844
 appetitive olfactory conditioning
 procedure, 832–836
 aversive olfactory conditioning
 procedure, 828–832
 control assays for sensory acuity and
 locomotion, 839–840
 data analysis and statistics, 840
 materials, 816–819, 819t–820t
 methods, 820–829
 olfactory acuity, testing for, 839
 recipe, 843–844
 shock acuity assay, 840
 sugar acuity, measuring, 840
 T-maze (see T-maze apparatus)
 troubleshooting, 840–842
- Clearance of damaged axons, 32–33,
 35–38, 43–44, 47
- ClockLab, 866
- Clock neurons, 432, 461, 464, 574, 582,
 618, 869, 873
- Clonal imaging of mitochondria in
 dissected fly wing (protocol),
 636, 642–647, 644f, 646f
 materials, 642–643
 method, 643–646, 644f, 646f
 quantitative analysis, 645–646, 646f
 sample preparation, 643–644
 troubleshooting, 646–647
- Co-immunoprecipitation (Co-IP), 127, 146
- Collection, fixation, and antibody staining
 of *Drosophila* embryos
 (protocol), 15–24
 antibody staining, 20–21
 discussion, 22
 egg collection cages, setting up, 18–19
 examination of stained embryos, 21, 22f
 fixing embryos, 19–20
 materials, 15–18, 17f
 method, 18–22, 22f
 recipes, 23–24
- Color bias, adjusting for, 837–838
- comatose*, 361
- commis sureless (comm)*, 5
- Competitive mating assay, 708, 717,
 718–719
- Complexin, 361
- Computer and software, for
 electrophysiology studies, 364
- Conductance (g), 350b
- Confocal microscopy, 10–11, 11f
 clonal imaging of mitochondria in
 dissected fly wing, 644–645
 ex vivo brain imaging, 608
 imaging neuropeptide release, 572
 imaging of learning-induced plasticity,
 588–589
 imaging presynaptic calcium influx,
 424–426, 425f
 scanning configuration, 572
 spinning disk, 572, 589
 transcriptional GECIs studies, 629
- Connectin (Con), 162
- Connectome, 209, 262
- Cornmeal fly media (recipe), 843–844
- Cornmeal–yeast *Drosophila* food (recipe),
 773, 779, 786
- Coupled colorimetric assay, 660
- Courtship behavior
 balancing motivation with other drives,
 709
 conditioning/suppression assays
 (protocol), 723–729, 726f
 courtship ritual as a behavioral duet,
 705–707, 706f
 female postmating behaviors, 706f,
 707–708
 female responses during, 706f, 707
 modulation of male behaviors, 708–709
 single-pair courtship and competitive
 assays (protocol), 714–722,
 715f
 vision, contribution of, 720
- Courtship chambers
 for audio experiments, 731, 731f
 cleaning, 717, 741
 fabrication, 715–716, 715f, 724, 737
- Courtship conditioning/suppression assays
 (protocol), 723–729, 726f
 basic conditioning assay, 725
 collecting male flies, 724
 conditioning using decapitated virgin
 female flies, 725
 data analysis, 726–727, 726f
 materials, 723–724
 method, 724–727, 726f
 satiety and recovery assays, 725
 spaced training assay, 725–726
 trainer females, generating, 724
 troubleshooting, 727–728
- Courtship song, 706–707, 720, 730–735,
 731f
- Courtship suppression, 708, 723
- Crawling
 imaging, 221
 kinematic data, 221–222
 studying larval in agarose channels
 (protocol), 214–226, 218f
 types of movements, 207
- The Crayfish* (Huxley), 158
- CRISPR, 323–341
 introduction, 323–325, 324f
 protocol, 326–341
 system components, 323–324, 324f
- CRISPR alleles in *Drosophila*, generating
 (protocol), 326–341
 dsDNA donor design, 333–334
 experimental strategy, 328–330
 generation of flies with edited alleles in
 their germlines, 335
 gRNA cloning, 331–332
 gRNA target site selection, 330–331
 identification of candidate gene edits—
 molecular screening,
 335–337, 336f
 identification of candidate gene edits—
 visible screening, 337–338
 materials, 326–328
 method, 33f, 328–338, 336f
 recipes, 340–341
 ssDNA donor design, 332–333, 333f
 troubleshooting, 339
- Cryosectioning
 for peripheral glial studies, 126–127
 whole-larva (protocol), 139–145,
 141f–142f
- Ctrax, 718, 759
- Culture of larval and pupal *Drosophila*
 dendritic arborization
 neurons (protocol), 105–114,
 106f, 108f, 110f
 culture of larval da neurons, 107–109,
 108f
 culture of pupal c4da neurons,
 109–110, 110f
 early larva cell collection, 107
 isolation of larvae, 107
 materials, 105–106
 method, 106–110, 108f, 110f
 microscopy, 109, 110
 plating and culture, 110
 plating culture, 108–109

- pupa dissection, 109–110
 reagent preparation, 107, 109
 recipes, 111–114
 third-instar larva cell collection, 107–108, 108f
 troubleshooting, 110–111
- Current (I), 350b
 Current clamp recording, 344t, 345
 Cut, 70–71
 Cuticular hydrocarbon (CH) pheromones, 849
 Cyclic AMP (cAMP) reporters, 588
- D**
- DAM. *See Drosophila* activity monitors (DAMs)
 DAM5H, 865
 DAM5M, 865, 867, 890–894, 892f–893f, 896, 904
 DAMFileScan, 867, 886, 897
 da neurons. *See* Dendritic arborization neurons
 Deaf flies, generating, 720
 DeepLabCut, 483
 Defective proboscis response (Dpr) proteins, 162
- Degeneration
 peripheral nerve crush in *Drosophila* larvae (protocol), 41–50, 43f, 45f
 Wallerian, 32, 35, 37f, 38, 41, 47, 61
- Dendrite arbor
 about, 67–68
 adult, 70
 DeTerm use for automated dendrite arbor terminal counts (protocol), 115–120, 116f, 118f–119f
 diversity, 68–70
 filleting and immunostaining of larvae to visualize dendritic arborization neuron dendrite arbor (protocol), 84–91, 86f–87f
 modes of growth, 73–74
 pattern formation, 74
 transcriptional control of subtype-specific morphology, 70–71
- Dendrite branch formation, 71–72
- Dendrite differentiation in dendritic arborization neurons, 67–120
 background, 67–68
 culture of larval and pupal *Drosophila* dendritic arborization neurons (protocol), 105–114, 106f, 108f, 110f
 dendrite branch formation, 71–72
 dendrite self-avoidance and tiling, 72–73
- DeTerm use for automated *Drosophila* dendrite arbor terminal counts (protocol), 115–120, 116f, 118f–119f
- different modes of dendrite arbor growth, 73–74
- filleting and immunostaining of larvae to visualize dendritic arborization neuron dendrite arbor (protocol), 84–91, 86f–87f
- morphological and functional diversity of dendritic arborization (da) neurons dendrite arbors, 67–68, 68t, 69f
- mosaic analysis with repressible cell marker (MARCAM) clone generation in dendritic arborization neurons (protocol), 79–83
- mounting of embryos, larvae, and pupae for live *Drosophila* dendritic arborization neuron imaging (protocol), 92–104, 94f, 96t, 97f–99f
- transcriptional control of subtype-specific dendrite arbor morphology, 70–71
- Dendrite-partitioning module, 72
- Dendritic arborization neurons
 adult, 70
 dendrite differentiation (protocol), 67–120
- DeTerm use for automated dendrite arbor terminal counts (protocol), 115–120, 116f, 118f–119f
- diversity, 68–70, 68t, 69f
- filleting and immunostaining of larvae to visualize dendritic arborization neuron dendrite arbor (protocol), 84–91, 86f–87f
- mosaic analysis with repressible cell marker (MARCAM) clone generation (protocol), 79–83
- mounting of embryos, larvae, and pupae for live *Drosophila* dendritic arborization neuron imaging (protocol), 92–104
- nociceptor, 764, 769
- Density assay for body fat determination in larvae (protocol), 661, 663–670, 669f
- data analysis, 667
 discussion, 668–669, 669f
 egg collection, 665–666
 experimental food preparation, 665
 fat level determination, 666–667
- grape plate preparation, 664–665
 larval transfer to experimental food, 666
 materials, 663–664
 method, 664–667
 recipes, 669–670
 troubleshooting, 667–668
- DeTerm use for automated *Drosophila* dendrite arbor terminal counts (protocol), 115–120, 116f, 118f–119f
- detection threshold optimization, 117
- discussion, 119
- exporting results, 118
- image quality improvement, 117
- manual correction, 118–119
- materials, 115
- method, 116–119, 118f–119f
- parameter tuning, 117
- preprocessing, 116–117
- providing a mask, 118
- software, 116
- Development, optic lobe, 261–278
- Developmental homeostasis, 406–407
- dFB (dorsal fan-shaped body), 468, 870, 874
- Differential interference contrast (DIC) microscopy, 10, 11f
- Digdata, 365
- Digital cameras, 571
- Dilp2–GFP, 572–573
- Dipping H₂O-immersion objective, 570
- Discs, 159
- Disposal of laboratory waste, 909–910
- Dissection
 adult brains, 273–274, 274f
 adult brains for patch-clamping, 448–454, 449f
 adult for in vivo imaging, 599
 body wall dissection tools and techniques (protocol), 168–178, 171f, 173f, 175f
 brain and antennae samples, 301–302
 caudal cuticle for two-photon microscopy, 496f
 central and peripheral nervous system of larvae (protocol), 131–138, 133f
 dorsal cuticle for two-photon microscopy, 497f
 larval, 412
 larval brains, 272
 larval brains for patch-clamping, 442–447, 443f
 larval CNS, 250–251
 larval for proximity ligation assay (PLA), 149–150
 larval into fillets, 132–134, 133f
 late-embryo dissection technique, 172–174, 173f

Index

- Dissection (*Continued*)
 live fly head for functional imaging
 (protocol), 516–520, 517f–518f
 pupal brains, 272–273, 273f
 third-instar larvae, 375, 418, 423
 ventral nerve cord dissection and microscopy of *Drosophila* embryos (protocol), 25–30
 wing, 644
- Dissection and immunolabeling of central and peripheral nervous system of larvae (protocol), 131–138, 133f
 direct visualization of fillets, 134
 dissection of larvae into fillets, 132–134, 133f
 immunolabeling of fillets, 134–135
 material, 131–132
 method, 132–135, 133f
 preparation for mounting, 135, 135f
 recipes, 137–138
 troubleshooting, 136
- Dissection of adult brains for patch-clamping neurons (protocol), 448–454, 449f
 discussion, 451–452
 materials, 448–449
 method, 449–451, 449f
 recipes, 452–454
 troubleshooting, 451
- Dissection of larvae into fillets, 132–134, 133f
- Dissection of live fly head for functional imaging (protocol), 516–520, 517f–518f
 fly chamber preparation, 517–518
 materials, 516–517, 517f
 method, 517–519
 mounting fly into fly chamber, 518–519, 518f
 opening head to expose optic lobes, 519
 recipe, 520
 troubleshooting, 519–520
- Dissection of wandering larval brains for patch-clamping neurons (protocol), 442–447, 443f
 discussion, 445
 materials, 442–443
 method, 443–444, 443f
 recipes, 445–447
 troubleshooting, 444–445
- Dissociation solution (recipe), 111
- Divider chamber assay, 858f, 858t, 859–861
- DNA extraction, by
 phenol–chloroform–isoamyl alcohol method, 305
- DNA fragmentation for ChIP, 297, 303, 306
- Doe, Chris Q., 2
- Dominance, 847–848
- Dopaminergic neurons, 634–635, 686–688, 687f, 747, 870
- Dorsal fan-shaped body (dFB), 468, 870, 874
- Dpr-interacting proteins, 162
- Driving force, 357–358, 358f
- Drosophila* activity monitors (DAMs)
 analysis of sleep and circadian rhythms from DAM data using SCAMP (protocol), 896–905
 modern, 865
 for monitoring multiple flies, 866
 neural stimulation during DAM-based studies of sleep and circadian rhythms (protocol), 879–889
 positional preference analysis using multibeam activity monitors (protocol), 890–895
- Drosophila* antifungal agent–ethanol solution (recipe), 669
- Drosophila* apple juice–agar plates (recipe), 82, 102, 111–112, 224, 234, 244, 258, 293, 743
- Drosophila* artificial hemolymph (recipe), 603, 610
- Drosophila* ChIP dissection buffer (recipe), 307
- Drosophila* cornmeal–yeast food (recipe), 779, 786, 888
- Drosophila* defined medium (DDM2) (recipe), 112
- Drosophila* insulin-like peptide (Dilp), 161
- Drosophila* population monitors (DPMs), 866
- Dscam1, 73–74
- dTRPA1, 764–765, 871–874
- DuoLink In Situ Orange, 147
- E**
- ECDs (extracellular domains), 186, 188–190
- Egg-collection apple juice–agar plates (recipe), 780, 786
- Egg collection baskets, 16, 17f
- Egg collection cages, 16, 17f, 18–19
- Egg laying, 738–739
- Eggs
 collection, 665–666
 laying behaviors, 707–708
- EJP (excitatory junction potential), 357, 379, 384
- Electrical circuits, 350, 351b
- Electrical terminology, 350b
- Electrodes, fabricating, 394–399, 396f, 398f
- Electrophysiological recording from chemosensory sensilla, 536–538
- fabrication of electrodes for, 394–399, 396f, 398f
- from a “model” cell (protocol), 368–373, 369f–371f
- from olfactory sensilla (protocol), 545–557, 548f, 550f–551f, 554f
- Electrophysiological recording from a “model” cell (protocol), 368–373, 369f–371f
 bridge balance, 368, 370–371, 371f
 capacitance compensation, 368–370, 370f
 materials, 368
 methods, 369–372, 370f–371f
 troubleshooting, 372–373
- Electrophysiology
 equipment setup for whole-brain recordings, 478–480, 480f
 patch-clamping (*see* Patch-clamping)
 sharp, 430–431, 431f
 to study homeostatic plasticity at NMJ, 401–427
 whole-brain during sleep and wake, 467–508
- Electrophysiology equipment, 362–365, 362t, 363f, 365f
- Electrophysiology rig, wiring diagram of, 365f
- Embryo
 collection, 172, 249, 290, 777, 783
 collection, fixation, and antibody staining of *Drosophila* embryos (protocol), 15–24
 in *Drosophila* life cycle, 2
 fixation, 19–20, 249–250
 heat shocking, 238–239
 late-embryo dissection technique, 172–174, 173f
 mounting, 94f, 95–96, 96t, 254–255, 255f
 sensorimotor circuit assembly, 205–260
 ventral nerve cord dissection and microscopy of *Drosophila* embryos (protocol), 25–30
- Embryo fixation solution (recipe), 258
- Embryonic axon guidance. *See* Axon guidance
- Empty neuron system, 538
- Energy homeostasis
 background, 659–661
 density assay for body fat determination in larvae (protocol), 663–670, 669f
- engrailed*, 6
- Epifluorescence microscope, 777–778
- Equilibrium potentials of individual ions, 355

- ERP (event related potential), 481–482, 484–485
- Ethanol behavioral responses, 745–762
background, 745–748
circuit basis of changes in locomotor activity (protocol), 751–761, 753f–754f, 758f
- Ethanol-induced changes in locomotor activity, circuit basis of (protocol), 751–761, 753f–754f, 758f
- ATR food medium preparation, 755–756
- data analysis, 758f, 759
- discussion, 759–760
- flyGrAM apparatus setup, 752–755, 753f–754f
- flyGrAM installation instructions, 755
materials, 751–752
methods, 752–759, 753f–754f, 758f
performing behavioral experiments, 756–759, 758f
recipe, 761
- Ethanol preference, 746
- Ethanol rapid iterative negative geotaxis (eRING) assay, 747
- Ethogram, 656, 709
- eve-Gal4*, 432, 458
- even-skipped*, 6, 255f
- Even-skipped protein, 255f
- Event related potential (ERP), 481–482, 484–485
- Excitatory/evoked postsynaptic potential (mEPSP) recording, 413–415
- Excitatory junction potential (EJP), 357, 379, 384
- Excitatory postsynaptic current (EPSC) recordings, 419, 420f
- Excitatory postsynaptic potential (EPSP), 345
- Extracellular domains (ECDs), 186, 188–190
- Extracellular fluid (ECF) (recipe), 506
- Extracellular recording, 343–345, 344t
- Ex vivo brain imaging (protocol), 605–611
data analysis, 609
explant preparation, 607–608
imaging, 608
imaging chamber, assembling, 606–607
materials, 605–606
method, 606–609
recipe, 610
stimulus application, 608–609
troubleshooting, 609–610
- Eye
calcium imaging of neural activity in photoreceptors, 509–530
structure, 261
- F**
- Faas, 866
- Fabrication of electrodes for electrophysiological recording, 394–399, 396f, 398f
- focal electrodes, manufacturing, 397–399, 398f
- materials, 394–395
- method, 395–399, 396f, 398f
- microelectrodes, manufacturing, 395–396
- suction electrodes, manufacturing, 296–397, 397f
- troubleshooting, 399
- FAP (fluorogen-activating protein), 573–574, 574f, 576–582
- Fasciclin 3 (Fas3), 159–160, 264
- Fat body, 660
- Fate specification, and larval optic lobe, 263–266
- Fat regulation, 659–660
- Feed-forward circuit motifs, 206
- Female fertility, 739
- Female postmating behaviors
overview, 706f, 707–708
protocol, 736–744, 741f
- Female postmating behaviors (protocol), 736–744, 741f
collection and rearing of flies, 737–738
egg laying, 738–739
female fertility, 739
materials, 736–737
mating plug ejection, 740–741
method, 737–741
ovulation, 738
postmating receptivity, 739, 741f
recipe, 743
remating, 739–740
testing and data analysis, 738–741
troubleshooting, 741–742
- Females
aggressive behavioral patterns, 846–848, 846t, 847f
mate-choice behavior, 706f, 707–708
postmating behaviors, 706f, 707–708, 736–744, 741f
responses during courtship, 706f, 707
- FicTrac, 499
- Fighting. *See* Aggression
- Filleting and immunostaining of larvae to visualize dendritic arborization neuron dendrite arbor (protocol), 84–91, 86f–87f
antibody staining, 87–88, 87f
larva dissection, 85–87, 86f
materials, 84–85
method, 85–89, 86f–87f
- mounting and storage, 88–89
preparation of sylgard-coated petri dish, 85
recipes, 90–91
troubleshooting, 89–90
- Fillets
direct visualization of, 134
dissection for peripheral glial studies, 126, 132–134, 133f
immunolabeling of, 134–135
proximity ligation assay (PLA) for fillets of larvae (protocol), 146–155
- Filter wheels, 571
- Fixation
brain dissections, 274
for ChIP studies, 303, 304f, 306
embryo, 19–20, 249–250
- Fixation solution for *Drosophila* larvae (recipe), 137, 145, 154
- Fixative (0.5%–1% formaldehyde) (recipe), 307
- Fixative for *Drosophila* embryos (recipe), 23
- FixTRAX, 709, 716, 718
- Flamindo, 588
- Flamingo, 72–73
- Fluorescence imaging lifetime microscopy-FRET (FLIM-FRET), 588
- Fluorescence resonance energy transfer (FRET), 127, 146
- Fluorescent in situ hybridization chain reaction for RNA in embryonic and larval central nervous system (protocol), 246–260, 248f, 251f–252f, 255f
analysis, 257
confocal imaging, 256
discussion, 256–257
embryo collection, 249
embryo fixation, 249–250
HCR staining, 251–253, 251f–252f
immunofluorescence staining, 254
larval CNS dissection and fixation, 250–251
larval CNS mounting, 255–256
materials, 246–249, 248f
method, 249–256, 251f–252f, 255f
mounting of embryos, 254–255, 255f
probe set design, 257
recipes, 258–260
troubleshooting, 256
- Fluorogen-activating protein (FAP), 573–574, 574f, 576–582
- Fly body length, 867
- Fly chamber
mounting fly into, 518–519, 518f
preparation, 517–518

Index

- Fly food (recipe), 39, 48, 56, 225, 235, 245, 761, 808
- Fly Group Activity Monitor (flyGrAM), 745, 748, 751–760
 apparatus setup, 752–755, 753f–754f
 installation instructions, 755
 performing behavioral experiments using, 756–759, 758f
- Fly saline (recipe), 520, 526
- FlyTracker, 709, 716, 718, 759, 861
- Focal electrodes, manufacturing, 397–399, 398f
- Focal recording of synaptic currents from single boutons at larval NMJ (protocol), 390–393
 materials, 390–391
 method, 391–392
 recipe, 393
 troubleshooting, 392
- Fold enrichment method, 298, 317, 320–321
- Food deprivation, 833, 834f
- Food preference, analysis of, 890–894
- Founder cells, 158
- Frazzled, 5, 8, 8f
- FRET (fluorescence resonance energy transfer), 127, 146
- Functional imaging
 dissection of live fly head for (protocol), 516–520, 517f–518f
 of learning-induced plasticity, 583–611
 from photoreceptors (protocol), 521–526, 522f, 524f
- Functional imaging from photoreceptors (protocol), 521–526, 522f, 524f
 assembling synchronized image-acquisition and visual stimulation system, 522–523, 522f
 finding GCaMP-expressing cells, 523
 materials, 521–522
 method, 522–525, 522f, 524f
 recipe, 526
 troubleshooting, 525
 in vivo calcium imaging of light-evoked neural activities, 523–525
- Functional imaging of learning-induced plasticity, 583–611
 with genetically encoded fluorescent reporters, 585–589, 587t
 in vivo imaging of olfactory, 594–604, 596f
- Fusion-competent myoblasts, 158
- ### G
- Gaboxadol, 870
- Gal4 drivers
 for imaging neuropeptide release, 569, 572
 lines for labeling glial cells, 125, 126t
 in mitochondrial dynamics studies, 637
 neural stem cells, 281–284, 282t, 292–293
 patch-clamping studies, 432
- Gal4–UAS system
 aggression studies, 850
 calcium imaging during sleep and wake, 489, 490f, 490t
 cell ablation techniques for larval neuromuscular system (protocol), 199–202, 201f
 in energy balance studies, 660, 668–669
 generation of CaLexA-ready flies, 626–627
 generation of TRIC-ready flies, 627
 MCFO technique and, 236–238, 243
 peripheral glia studies, 125
 sleep studies, 868
- γ -aminobutyric acid (GABA), 588, 660, 850–851, 870
- Gap junctions, 125
- GCaMP, 870
 for calcium imaging in photoreceptors, 511, 523–525, 524f, 529–530, 530f
 imaging in recording during sleep and wake, 489–490, 490f, 490t, 501–504
 imaging of learning-induced plasticity, 585–587
 tagged neuropeptides, 573
 in vivo imaging of olfactory learning-induced plasticity, 600–602
- GCaMP6, 586, 587t, 614
- GCaMP6f, 489–490, 586
- GCaMP6m, 227–228, 231, 232f, 234
- Gene editing, CRISPR, 323–341
- GeneSwitch system, 870
- Genetically encoded calcium indicators (GECIs), 346
 advantages and disadvantages, 510–511, 615t
 for calcium imaging in photoreceptors, 510–513
 dual-FP vs. single-FP, 510–511
 FRET-based, 585–586, 614
 imaging of learning-induced plasticity, 585–587, 587t
 overview, 614–615
 single-wavelength, 586
 transcriptional, 613–631, 615t
 whole-brain recordings during sleep and wake, 489–490, 490t
- Genetically encoded fluorescent reporters, 585–589, 587t
- Genetically encoded voltage indicators (GEVIs), 346, 511, 588
- Geotaxis, 747
- Gigaohm seals (giga-seal), 345, 430, 461
- Glia
 of CNS, 122
 overview, 121–122
 perineurial, 125
 of PNS (*see* Peripheral glial cells)
 protease treatment for removal of superficial glia, 456–459
 subperineurial, 124–125
 wrapping, 122, 123f, 124
- Glial sheath, removal of, 458
- Gluing brains to coverslips, 445, 452
- Glutamate receptors (GluRs), 158–159, 357
 calcium-permeable, 613
 philanthotoxin-433 inhibition of, 404, 410, 412
 PHP through loss of function, 403–404, 404f
- Glutamatergic neurons, 158, 160, 850–851
- Glycerol (70%, v/v, in PBS) (recipe), 23, 30
- Glycerol stock solutions (recipe), 137, 155
- Goldman–Hodgkin–Katz (GHK) equation, 355–356
- Goro neurons, 765
- Grape agar plates (recipe), 176, 203
- Graph theoretical analysis, 486, 505
- Green fluorescent protein (GFP)
 imaging neuropeptide release, 569, 572–573, 576–582, 578f
 pH-sensitive variants, 573
- Guidepost cells, 6
- Guide RNA (gRNA)
 cloning, 331–332
 generating CRISPR alleles in *Drosophila* (protocol), 327–335, 338
 overview, 323–324, 324f
- Gustation
 blocking, 720
 pheromones, 706
- Gustatory receptors (Grs), 534–536, 691–692
- Gustatory system
 anatomy, 532–534, 533f
 chemosensory coding principles, 539–540
 electrophysiological recordings from chemosensory sensilla, 537–538
 recording from taste sensilla (protocol), 558–568, 561f–562f, 566f
- Gut microbiome, influence on aggression, 850–851
- ### H
- H₂O-immersion objective, 570
- Hairpins, fluorescent HCR amplifier, 257

- Hazardous chemicals, properties of, 911–912
- HDR (homology-directed repair), 323–325, 324f, 327–329, 332, 333f, 334–335
- Head-casts, 790–791
- head involution defective*, 198
- Heat shock
 death following, 292
 of embryos, 238–239
 of larvae, 291–292
- Hebbian types of plasticity, 403
- Hemineuromere, 208
- Hemisegment, 208
- hid*, 720
- High-K⁺ (100 mM) saline for neuronal stimulation (recipe), 610
- High numerical aperture objective, 570–571, 570f
- HL-3 (recipe), 582
- HL3.1 solution (recipe), 90
- HL-3 saline (variable Ca²⁺) (recipe), 382, 388, 393
- Homeostatic challenges, 401–402
- Homeostatic compensation, 402f
- Homeostatic plasticity at neuromuscular junction (NMJ), 401–427
 eliciting PHP at larval NMJ (protocol), 410–416
 presynaptic calcium influx at larval neuromuscular junction (protocol), 422–427, 425f
 presynaptic homeostatic depression (PHD), 402f, 404, 406
 presynaptic homeostatic potentiation (PHP), 402–404, 402f, 404f, 405t–407t
 readily releasable synaptic vesicle pool (RRP) at larval NMJ (protocol), 417–421, 420f
- Homogenization buffer for *Drosophila* (recipe), 112
- Homology-directed repair (HDR), 323–325, 324f, 327–329, 332, 333f, 334–335
- Humidity chamber, 150, 152
- Huxley, Thomas, 158
- 5-hydroxytryptamine (5HT), 851
- I**
- ImageJ, 118–119, 231, 241, 527–528, 571, 580–581, 601–602, 651–652
- Imaging
 CaLexA and TRIC signals in dissected fly CNS (protocol), 624–631
 ex vivo brain imaging (protocol), 605–611
 larval behavior in agarose channels, 217–219, 218f, 221–222
 of learning-induced plasticity, 583–611
 neural activity in intact, semirestrained larvae (protocol), 227–235, 230f, 232f
 neuronal activities, 345–346
 neuropeptide release, 569–582
 synaptic vesicle cycling, 346
 in vivo of olfactory learning-induced plasticity, 594–604, 596f
- Imaging CaLexA and TRIC signals in dissected fly CNS (protocol), 624–631
 generation of flies, 626–627
 image analysis and quantification, 629
 immunohistochemistry and imaging, 627–629
 materials, 624, 625t, 626
 method, 626–629
 troubleshooting, 629–630
- Imaging neural activity in intact, semirestrained larvae (protocol)
 discussion, 233–234
 image analysis, 231–232, 232f
 imaging neural responses in a semi-intact preparation, 230–231, 230f
 larvae preparation, 228–229
 materials, 227–228
 method, 228–232, 230f, 232f
 recipes, 234–235
 sound stimulus setup, 229–230
 troubleshooting, 232–233
- Imaging neuropeptide release, GFP and FAP (protocol), 576–582, 578f
 discussion, 582
 materials, 576–582, 578f
 method, 577–581
 recipe, 582
 troubleshooting, 581
- Immobilizing second-instar *Drosophila* larvae for imaging and surgery using the larva chip (protocol), 51–57, 53f–54f
 discussion, 55
 imaging the larvae, 55
 immobilizing larvae in larva chip, 53–54
 larva chip preparation, 52–53, 53f–54f
 materials, 51–52
 method, 52–55, 53f–54f
 recipes, 56
- Immunofluorescence
 collection, fixation, and antibody staining of *Drosophila* embryos (protocol), 20–21
 fluorescent in situ hybridization chain reaction for RNA, 254
 immunohistochemistry *versus*, 10–12
 larval CNS, 240
- Immunohistochemistry
 collection, fixation, and antibody staining of *Drosophila* embryos (protocol), 20–21
 dendritic arborization (da) neurons in larval fillets, 87–88, 87f
versus immunofluorescence, 10–12
 of larval body wall NMJ preparations (protocol), 179–185, 181f–182f
 transcriptional GECIs studies, 627–628
- Immunolabeling
 of central and peripheral nervous system of larvae (protocol), 131–138, 133f
 proximity ligation assay (PLA) for fillets of larvae, 150
 whole-larva (protocol), 139–145, 141f–142f
- Immunostaining to study optic lobe development (protocol), 270–278, 273f–274f, 276f
- Independent component analysis, 484
- Inebri-actometer, 747
- Inebriometer, 747
- Inhibitory postsynaptic potential, 345
- Inositol 1,4,5-triphosphate receptors (IP₃Rs), 613
- Insect behavior, 655
- Institutional Safety Office, 907
- Integrated information theory, 486
- Intermediate neural progenitors (INPs), 279, 282–284, 283f, 287
- Interneuron, 208
- Intracellular recording, 344t, 345
- In vivo imaging of olfactory learning-induced plasticity (protocol), 594–604, 596f
 data analysis, 601
 fly preparation/dissection, 599
 heating element, building, 597
 imaging apparatus construction, 596f, 597–598, 599–600
 materials, 594–595
 method, 596f, 597–601
 odor-delivery apparatus, 598–599
 recipe, 603
 troubleshooting, 601–602
- Ion channels, and membrane potential, 356–357
- Ionic electromotive force (EMF_{ion}), 357–358
- Ionotropic receptors (Irs), 534–535
- J**
- JAABA, 709, 716, 759, 861
- jGCaMP7, 586, 587t, 614

Index

- jGCaMP8, 587t, 601–602
 Johnston's organ, 849
 Juice agar plates for *Drosophila* embryo collection (recipe), 23
- K**
 Katz, Bernard, 359
 KD recombinase, 292–293
 Kenyon cells, 584, 686–688, 687f
 Knot, 71
- L**
 Labellar sensilla, recording from, 562–563, 562f
 Lamina, optic lobe
 neuron specification, 264–265
 structure, 262–263
 LAMs (locomotor activity monitors), 866
 Large lateral ventral neurons (LLNs), 432, 458, 459f, 464
 Larva chip, 51–55, 53f–54f
 Larva dissection
 dissection and immunolabeling of central and peripheral nervous system of larvae (protocol), 131–138, 133f
 Larvae
 behavior in agarose channels (protocol), 214–226, 218f
 body organization of, 206–207, 207f
 brain dissection, 272
 cell ablation techniques for larval neuromuscular system (protocol), 198–204
 CNS anatomy, 208–209, 208f
 collection, 216–217
 culture of larval and pupal *Drosophila* dendritic arborization neurons (protocol), 105–114, 106f, 108f, 110f
 density assay for body fat determination, 661, 663–670, 669f
 direct visualization of fillets, 134
 dissection (*see* Larval dissection) in *Drosophila* life cycle, 2
 filleting and immunostaining to visualize dendritic arborization neuron dendrite arbor (protocol), 84–91, 86f–87f
 fillets for peripheral glial studies, 126, 132–134, 133f
 fixation for immunolabeling, 174–176, 175f
 heat shock, 291–292
 immobilizing second-instar for imaging and surgery using the larva chip (protocol), 51–57, 53f–54f
 immunolabeling of fillets, 134–135
 laser microsurgery in *Drosophila* larvae using the MicroPoint ablation system (protocol), 58–66, 60f–62f
 learning and memory, 671–689
 mounting (*see* Larval mounting)
 neural fate specification in optic lobe, 263–266
 neuromuscular junction (NMJ), 157–204
 neurons and circuits, 208–209
 nociception, 763–787
 overview of stage, 790–791
 peripheral glial cells, 121–155
 peripheral nerve crush in *Drosophila* larvae (protocol), 41–50, 43f, 45f
 proximity ligation assay for fillets of larvae (protocol), 146–155
 proximity ligation assay use to visualize colocalization of proteins at larval NMJ (protocol), 193–197, 195f
 sensorimotor circuit assembly, 205–260
 tracking navigation, 789–809, 790f
 washing and drying, 799f
 whole-larva cryosectioning and immunolabeling of larvae (protocol), 139–145, 141f–142f
 Larval behavior in agarose channels (protocol), 214–226, 218f
 cellular activity data, 222
 channel design, 223
 channel mold manufacturing, 223–224
 collection of larvae, 216–217
 discussion, 220–224
 generating linear channels, 222–223
 imaging, 216–217, 217–219, 218f, 221–222
 kinematic data, 221–222
 materials, 214–215
 method, 215–219, 218f
 negative channel molds, production of, 216
 positive channel mold creation using etching/cutting, 224
 positive channel mold creation using photolithography, 223
 recipes, 224–225
 troubleshooting, 219–220
 Larval dissection, 412
 brain for optic lobe studies, 272
 CNS, 239–240, 250–251
 into fillets, 132–134, 133f
 for immunolabeling, 174–176, 175f
 for patch-clamping brain neurons, 442–447, 443f
 third-instar larvae, 375, 418, 423
 Larval external saline (recipe), 445, 465
 Larval internal saline (recipe), 441, 465–466
 Larval mounting
 brain sections, 276–277
 CNS, 241, 255–256
 mounting filleted, 135, 135f
 mounting for live *Drosophila* dendritic arborization neuron imaging (protocol), 92–104
 Larval neural stem cell markers, 281–284, 282t, 283f
 Laser etching, creating channel molds using, 224
 Laser microsurgery in *Drosophila* larvae using the MicroPoint ablation system (protocol), 58–66, 60f–62f
 calibrating laser intensity, 63
 discussion, 65
 materials, 58–59
 method, 59–64, 60f–62f
 refocusing the laser, 63–64
 replacing the laser dye, 61–63, 62f
 troubleshooting, 64
 using MicroPoint laser to carry out surgical injury or ablation, 59–61, 60f–62f
 LB (Luria-Bertani) liquid medium (recipe), 340
 Learning
 classical conditioning, 811–844
 functional imaging of learning-induced plasticity, 583–611
 multisensory, 811, 813, 837
 nonreciprocal, 675, 675f
 olfactory associative, 583–584, 671–675
 Learning and memory in larvae, 671–689
 analysis of aversive olfactory–taste (protocol), 678–689, 680f–681f, 683f–684f
 assay for olfactory associative learning and memory, 671–675
 Leg sensilla, recording from, 563–564
 LFP (local field potential) activity, 468–469, 470f, 473, 475, 483–486, 865
 Life cycle, *Drosophila*, 2–3
 Lifesong, 716, 718
 Ligand-gated channels, 356–357
 Light sheet microscopy, 589
 Light source, choice of, 571
 Light stimulation. *See* Optogenetics

- Lineage analysis and birthdating of central complex lineages (protocol), 287–294, 288f
 discussion, 292–293
 materials, 287–289
 method, 289–291
 recipes, 293
 troubleshooting, 292
- Live imaging of mitochondria in intact fly wing (protocol), 636–637, 638–653, 650f
 live imaging steps, 651
 materials, 648–649
 method, 649–652
 quantitative analysis, 651–652
 sample preparation, 649–651, 650f
 troubleshooting, 652–653
- Live yeast paste. *See* Yeast paste
- LLNs (large lateral ventral neurons), 432, 458, 459f, 464
- Lobula, optic lobe, 263
- Lobula plate, optic lobe, 263
- Local field potential (LFP) activity, 468–469, 470f, 473, 475, 483–486, 865
- Locomotion
 peristalsis, 790–791
 rolling behavior, 763–765, 772–773, 775, 778, 781, 784–785
 tracking navigation behavior in odor gradients, 789–809, 790f
- Locomotor activity monitors (LAMs), 866
- Long-term memory (LTM), 812–813
- M**
- Machine-learning algorithms, 486
- Maggot learning, 671–689
- Malachite green, 573–574
- Males
 aggressive behavioral patterns, 846–848, 846t, 847f
 modulation of male reproductive behaviors, 708–709
- MARCA. *See* Mosaic analysis with repressible cell marker
- Mate-choice behavior in female flies, 706f, 707–708
- Material Safety Data Sheets (MSDSs), 907
- Mating plug, 707, 710, 736, 740–741
- Maxillary palp
 anatomy, 533, 533f
 recording from, 553
- MB (mushroom body), 584–585, 686–688, 687f, 864
- MBONs (mushroom body output neurons), 687–688, 687f
- MCFO (multicolor flip-out), 236–245, 239f, 242f
- Mdr65, 125
- MEAs (microelectrode arrays), 344
- Mechanical nociception, 764, 769–774, 771f
- Mechanical nociception assay in larvae (protocol), 769–774, 771f
 assay steps, 771–772
 discussion, 772–773
 fly husbandry and cross preparation, 770–772
 materials, 769–770
 method, 770–772, 771f
 recipe, 773
 von Frey filament, constructing, 770, 771f
- Mechanosensory neurons, 68–69, 534, 536, 565, 620
- Media containing agar or agarose (recipe), 340
- Medical Pathological Waste (MPW), 909
- Medulla, optic lobe
 neuron specification, 265–266
 structure, 262–263
- Membrane permeability
 ion channels and, 356–357
 relative, 358
 selective, 353
- Membrane potential
 action potentials, synaptic potentials, and driving force, 357–359, 358t
 for entire cell, 355–356
 equilibrium potentials of individual ions, 355
 generation of, 353
 membrane permeability and ion channels, 356–357
 Na⁺/K⁺ pump, 356
 physical basis for, 353–354, 354f
 recording, 375–377
 resting, 350–359, 354f
 reversal potential, 357–359
 selective membrane permeability, 353
 unequal distribution of ions between inside and outside of a cell, 353
- Memory
 anesthesia-resistant memory (ARM), 812
 aversive taste, 692–693
 classical conditioning, 811–844
 courtship conditioning/suppression assays, 725–726
 functional imaging of learning-induced plasticity, 583–611
 nonconsolidated, 813
 protein synthesis-dependent long-term memory (LTM), 812–813
- mEPSC (miniature excitatory postsynaptic current), 419
- mEPSP (miniature excitatory postsynaptic potential), 413–415
- Microelectrode arrays (MEAs), 344
- Microelectrode puller, 364
- Microelectrodes, manufacturing, 395–396
- Microforge, 397–398, 438
- Microgrinder, 398–399
- Micro lance, 498
- Micromanipulators, 364
- MicroPoint laser ablation system, 58–66, 60f–62f
- Microscopy
 components, 571
 confocal (*see* Confocal microscopy)
 for electrophysiology studies, 362–363
 fluorescence imaging lifetime microscopy-FRET (FLIM-FRET), 588
 high numerical aperture objective, 570–571, 570f
 imaging neuropeptide release, 569–582
 imaging of learning-induced plasticity, 588–589
 resolution, 139
 two-photon (*see* Two-photon microscopy)
 wide-field epifluorescence microscope, 569–570
- Miniature excitatory postsynaptic current (mEPSC), 419
- Miniature excitatory postsynaptic potential (mEPSP), 413–415
- Miniature synaptic potentials (minis), 384, 387
- miniSOG2, 199–202, 201f
- Mitochondrial DNA (mtDNA), 635
- Mitochondrial dynamics in adult axons, analysis of, 633–653
 background, 633–636
 clonal imaging in dissected fly wing (protocol), 636, 642–647, 644f, 646f
 live imaging in intact fly wing (protocol), 636–637, 638–653, 650f
 overview of methods, 636–637, 637t
 transgenic fly lines used to detect biochemical mitochondrial changes, 638t
 transgenic fly lines used to fluorescently label mitochondria, 637t
- Mitochondrial dysfunction, 634–635
- Mitochondrial transport, 635–637, 650f, 651–653
- Mitophagy, 634
- μManager, 571
- MMJ. *See* Neuromuscular junction

Index

- Modified HL-3 saline (recipe), 415, 421, 427
- Morphometric analysis of larval body wall NMJ preparations, 179–185, 181f–182f
- Mosaic analysis with repressible cell marker (MARCM)
- clone generation in dendritic arborization (da) neurons (protocol), 79–83
 - in mitochondrial dynamics studies, 633, 636, 643, 644f
- Mosaic analysis with repressible cell marker (MARCM) clone generation in dendritic arborization (da) neurons (protocol), 79–83
- genetic cross, 80
 - larva preparation, 80–81
 - materials, 79–80
 - method, 80–81
 - recipes, 82–83
 - screening for clones, 81
- Motor circuit, 205–260
- assembly, 205–260
 - Drosophila* as a study model of assembly, 206
 - imaging neural activity, 227–235, 232f
 - introduction, 205–206
 - larval neurons and circuits, 208–209
 - neural stem cells and neural diversity, 209–211, 210f–211f
- Motor neuron–muscle recognition, 161–162
- Motor neurons, types of larval, 160
- Mounting
- brain sections, 275–277, 276f
 - embryos, 93, 94f, 95–96, 96t, 254–255, 255f
 - fly into fly chamber, 518–519, 518f
 - larvae, 96, 97f
 - larval brain sections, 276–277
 - larval CNS, 241, 255–256
 - larval fillets, 88–89, 135, 135f
 - pupa, 98f, 99
- Mounting of embryos, larvae, and pupae
- for live *Drosophila* dendritic arborization neuron imaging (protocol), 92–104, 94f, 96t, 97f–99f
 - discussion, 100–101
 - embryo collection, 94–95
 - embryo mounting, 93, 94f, 95–96, 96t
 - larva mounting for live da neuron imaging, 96–97, 97f
 - larva preparation, 96
 - materials, 92–93
 - method, 93–100, 94f, 96t, 97f–99f
 - microscopy, 96–97
 - mounting, 96, 97f
 - mounting and imaging, 98f–99f, 99–100
 - pupa collection and staging, 97–98, 97f
 - pupa mounting for live da neuron imaging, 97–100, 97f
 - pupa preparation, 98–99, 98f
 - reagent preparation, 93–94
 - recipes, 101–103
 - troubleshooting, 100
- Movement. *See also* Locomotion
- larval body, 206–207
 - motor circuits (*see* Motor circuit)
 - somatosensory stimuli use, 207
- MPW (Medical Pathological Waste), 909
- MSDSs (Material Safety Data Sheets), 907
- mtDNA (mitochondrial DNA), 635
- Multibeam activity monitors (MB5s), 865
- MultiClamp Commander, 461
- Multicolor flip-out (MCFO), 236–245, 239f, 242f
- Multiplexing neural recordings, 512–513
- Multisensory conditioning, aversive and appetitive, 836–839
- adjusting for color bias, 837–838
 - LED assembly, 836, 837f
 - memory performance, calculating, 839
 - multisensory training, 838–839
 - preparing flies, 837
 - testing, 839
 - T-maze setup for, 837, 838f
- Multiwell chamber assay, 857, 858f, 858t, 859
- Mushroom body (MB), 584–585, 686–688, 687f, 864
- Mushroom body output neurons (MBONs), 687–688, 687f
- ## N
- Naka–Rushton function, 527, 529–530
- Na⁺/K⁺ ATPase, 356
- nanos-Cas9*, 329
- Nerst equation, 355
- Nerve crush, peripheral in *Drosophila* larvae (protocol), 41–50, 43f, 45f
- Netrin, 6–10, 8f, 73, 162
- Neural circuit formation, in optic lobe, 267
- Neural development
- in context of life cycle, 2–3
 - overview, 1–2
 - studying, 3
- Neural diversity, generation of, 209–211, 210f
- Neural fate specification, and larval optic lobe, 263–266
- Neural lineages, 209
- Neural stem cells
- generation of neural diversity, 209–211, 211f
 - intermediate neural progenitors (INPs), 279, 282–284, 283f, 287
 - larval markers, 281–284, 282t, 283f
 - overview, 279–280
 - temporal patterning, 283f
 - types of division, 280
- Neural stimulation during DAM-based studies of sleep and circadian rhythms (protocol), 879–889
- activity-monitoring experiments, 883–886
 - data-collection computer setup, 881–882
 - fly preparation, 882–883
 - incubator setup, 881
 - materials, 879–881
 - method, 881–887, 885f–887f
 - recipes, 888
 - recycling activity-monitoring tubes, 886–887
- Neuroblasts, 209
- Neurogenesis
- central complex lineages, 279–280
 - optic lobe, 261–262, 264, 267
 - overview, 1–2
- Neuromeres, 1
- Neuromodulators, and aggression regulation, 851
- Neuromodulatory neurons, 160–161
- Neuromuscular circuit development, 158–160, 159f
- Neuromuscular circuit map, 160–161
- Neuromuscular junction (NMJ)
- background, 157–158
 - body wall dissection: dissection tools and techniques (protocol), 168–178, 171f, 173f, 175f
 - cell ablation techniques for larval neuromuscular system (protocol), 198–204
 - degeneration at, 41–47
 - developmental homeostasis, 406–407
 - elaboration during larval development, 162–163
 - focal recording of synaptic currents from single boutons at larval NMJ (protocol), 390–393
 - growth signaling, 163
 - homeostatic plasticity at, 401–427
 - imaging neuropeptide release, 569–570, 572–573
 - immunohistochemistry and morphometric analysis of larval body wall NMJ preparations (protocol), 179–185, 181f–182f

- labeling cell surface proteins at larval NMJ using binding partner peptides (protocol), 186–192, 188f
 - larval, 157–204
 - presynaptic calcium influx at larval (protocol), 422–427, 425f
 - presynaptic homeostatic potentiation (PHP) elicitation at larval (protocol), 410–416
 - proximity ligation assay use to visualize colocalization of proteins at larval NMJ (protocol), 193–197, 195f
 - readily releasable synaptic vesicle pool (RRP) at larval NMJ (protocol), 417–421, 420f
 - synaptic electrophysiology of, 349–399
 - vertebrate, 157–158
 - voltage-clamp analysis of synaptic transmission at larval NMJ, 384–389, 387f
 - Neuronal activity
 - direct recording, 343–345
 - imaging, 345–346
 - intracellular calcium and, 613–614
 - Neuronal culture medium 1 (recipe), 112
 - Neuronal culture medium 2 (recipe), 113
 - Neuron specification in optic lobe
 - inner proliferation center (IPC), 266
 - lamina, 264–265
 - medulla, 265–266
 - Notch-dependent binary fate choices, 266
 - overview, 263–264
 - spatial patterning, 265, 266
 - temporal patterning, 265, 266
 - Neuropeptide release, imaging, 569–582
 - background, 569
 - digital cameras, 571
 - indicators in flies, 572–574, 574f
 - protocol, 576–582, 578f
 - wide-field epifluorescence microscope, 569–570
 - Neuropeptide-release reporters, 573
 - Neuropeptides, and aggression regulation, 851
 - Neurotransmitters, and aggression regulation, 849–851
 - NFAT (nuclear factor of activated T cells), 615–617, 616f
 - NHEJ (nonhomologous end-joining), 323–325, 324f, 328–329, 335, 338
 - Nicotinamide mononucleotide adenyltransferase (NMNAT), 32
 - Nicotinic acetylcholine receptors, 613
 - Nociception
 - in adults, 766
 - assaying behaviors during parasitoid wasp attack (protocol), 781–787
 - background, 763–764
 - chemical, 764
 - circuits, 765
 - in larvae, 763–787
 - mechanical, 764, 769–774, 771f
 - nociceptor neurons, 764
 - noxious stimuli, 764–765
 - optogenetic stimulation of escape behaviors in larvae (protocol), 775–780
 - parasitoid wasp attack, 765, 781–787
 - plasticity, 765–766
 - thermal, 764
 - Nociceptor neurons, 764
 - No mechanoreceptor potential C (NompC), 69
 - Nonhomologous end-joining (NHEJ), 323–325, 324f, 328–329, 335, 338
 - Nonreciprocal learning, 675, 675f
 - Normal goat serum (5%) in 0.1% PBST (recipe), 184, 191, 196
 - NoRMCorre, 233
 - Notch-dependent binary fate choices, in optic lobe, 266
 - Notch-Off daughter, 209
 - Notch-On daughter, 209
 - Noxious stimuli, 764–765
 - Nuclear factor of activated T cells (NFAT), 615–617, 616f
 - Numerical aperture, 570–571, 570f
- O**
- Octb2R receptor, 160–161
 - Octopamine, and aggression, 850–851
 - Odorant-binding protein (Obp), 534
 - Odorant receptors (Ors)
 - chemotaxis behavior and, 791
 - overview, 534–535
 - Odor gradients
 - Raspberry Pi Virtual Reality (PiVR) system study of larval chemotaxis with (protocol), 793, 795–809, 802f–806f
 - tracking navigation behavior of larvae in, 789–809, 790f
 - Ohm’s law, 351b, 372
 - Olfaction
 - during courtship, 720
 - ethanol behavior responses, 760
 - learning and memory, 811–814, 816–842
 - Raspberry Pi Virtual Reality (PiVR) system study of larval chemotaxis with odor gradients (protocol), 793, 795–809, 802f–806f
 - Olfactory acuity, testing for, 839
 - Olfactory associative learning and memory,
 - assay for, 671–675
 - aversive odor–taste learning, 673–674, 678–689, 680f–681f, 683f–684f
 - background, 671–675
 - nonreciprocal learning, 675, 675f
 - one-odor learning, 674
 - two-odor reciprocal aversive odor–high salt learning, 672–673
 - Olfactory sensilla, recordings from (protocol), 545–557, 548f, 550f–551f, 554f
 - data analysis, 554–555, 554f
 - discussion, 556
 - fly preparation for recording, 550–552, 551f
 - materials, 545–547
 - method, 547–555, 548f, 550–551f, 554f
 - odor cartridge preparation, 549
 - odor stimulus device, assembly of, 549
 - performing recordings, 553–554
 - recording from antennal sensilla, 552–553
 - recording from maxillary palp, 553
 - recording rig, assembly of, 549–550, 550f
 - troubleshooting, 555–556
 - tungsten electrode sharpener, 547–548, 548f
 - Olfactory sensory neurons (OSNs)
 - anatomy, 532
 - chemosensory coding principles, 538–539
 - electrophysiological recordings from chemosensory sensilla, 536–537
 - odor recognition, 534–535
 - receptive field, 791
 - Wallerian degeneration and clearance of olfactory receptor neuron axons following *Drosophila* antennal transection (protocol), 35–40, 37f
 - Olfactory system
 - anatomy, 532, 533f, 584–585
 - associative learning, 583–584
 - chemosensory coding principles, 538–539
 - electrophysiological recordings from chemosensory sensilla, 536–537
 - molecular biology of chemosensation, 534–536, 535–536

Index

- Olfactory system (*Continued*)
 recordings from olfactory sensilla
 (protocol), 545–557, 548f,
 550f–551f, 554f
 in vivo imaging of olfactory learning-
 induced plasticity, 594–604,
 596f
- Optic lobe
 axon guidance, 267
 immunostaining to study development
 (protocol), 270–278,
 273f–274f, 276f
 neural circuit formation, 267
 neural fate specification, 263–266
 opening fly head to expose for imaging,
 519
 overview, 261–262
 structure, 262–263, 264f
- Optic lobe development, immunostaining
 to study (protocol), 270–278,
 273f–274f, 276f
 dissections, 272–274, 273f–274f
 fixation, 274
 imaging and data analysis, 277
 materials, 270–271, 271t
 method, 272–277, 273f–274f, 276f
 mounting, 275–277, 276f
 recipes, 278
 staining, 274–275
- Optogenetic experiments
 ATR food medium preparation for,
 755–756
 creating virtual realities with, 791–792
 Raspberry Pi Virtual Reality (PiVR)
 system, 795
 sleep studies, 868–872, 869t
- Optogenetics approaches to sleep studies,
 868–872, 869t
 benefits of, 868–870
 limitations of, 870–871
 methodological considerations,
 871–872
 neural stimulation during DAM-based
 studies of sleep and circadian
 rhythms (protocol), 879,
 882–883, 885–886, 885f–886f
- Optogenetic sensors, 868–872
- Optogenetic stimulation of nociceptive
 escape behaviors in larvae
 (protocol), 775–780
 behavioral assay, 777–778
 behavior apparatus setup, 777
 discussion, 778–779
 embryo collection and larval culture,
 777
 fly husbandry and cross preparation,
 776
 materials, 775–776
 method, 776–778
- recipes, 779–780
- Orco, 720, 791
- Oscilloscope, 365
- OSNs. *See* Olfactory sensory neurons
- Ovulation, 738
- P**
- Pakin, 634–635
- PAM (protospacer-adjacent motif), 324,
 324f, 330–334, 339
- Paraformaldehyde (4%, w/v) in PBS
 (recipe), 90
- Parasitoid wasp attack, assaying
 nociception behaviors during
 (protocol), 765, 781–787
 behavioral assay and analysis, 784
 culturing wasp colonies, 782–783
 discussion, 785
Drosophila embryo collection and larval
 culture, 783–784
 materials, 781–782
 method, 782–784
 recipes, 785–786
- Parkinson’s disease, 634–635
- PATCH-1U model cell, 463
- Patch clamping, 345
 background, 429–430
 difficulty of technique, 430–431
 meaning of, 429–430
 neuron types to be patched, 431–432
 sharp electrophysiology compared,
 430–431, 431f
- Patch-clamping brain neurons, 429–466
 dissection of adult brains (protocol),
 448–454, 449f
 dissection of wandering larval brains
 (protocol), 442–447, 443f
 introduction, 429–434
 preparation of pipettes and pipette-
 filling devices (protocol),
 435–441
 protocol, 455–466
- Patch-clamping brain neurons (protocol),
 455–466
 discussion, 464
 materials, 455–456
 method, 456–462, 459f
 patch clamping, 459–462
 protease treatment for removal of
 superficial glia, 456–459
 recipes, 464–466
 troubleshooting, 462–463
- Patch-clamping pipettes and pipette-filling
 devices, preparation of
 (protocol), 435–441
 changing filament on Sutter horizontal
 puller, 438–439
 discussion, 439–440
- homemade pipette-filling devices, 438
 materials, 435–441
 method, 436–439, 436t–437t
 polishing pipettes, 437–438
 pulling pipettes, 436–437, 437t
 recipes, 440–441
- Patch pipette dye (recipe), 466
- Pathetic, 74
- PBS (10× pH 7.4) (recipe), 278
- PBS 10× (recipe), 244, 258
- PBS containing 0.1% Triton X-100 (PBT)
 (recipe), 24
- PBST (PBS with 0.1%, v/v Triton X-100)
 (recipe), 245
- PBS with 0.1% (v/v) Triton X-100 (PBST)
 (recipe), 83, 90, 103, 113, 259
- PBS–yeast paste (recipe), 670
- pCLAMP, 464
- PdF (pigment-dispersing factor), 432
- Pdm1 and 2, 70
- PDMS (polydimethylsiloxane) larva chip,
 51–55, 53f–54f
- pegRNAs (prime editing gRNAs), 325
- PER (proboscis-extension response), 691,
 692, 696–697, 696f, 701f,
 702–703
- Percent input method, 298, 317, 320–321
- Perineurial glia, 125
- Peripheral glial cells, 121–155
 dissection and immunolabeling of
 central and peripheral
 nervous system of larvae
 (protocol), 131–138, 133f
 Gal4 driver lines for labeling glial cells,
 125, 126t
 methods enabling study of, 125–128
 nervous system and, 122–125, 123f
 proximity ligation assay (PLA) for fillets
 of larvae (protocol), 146–155
 whole-larva cryosectioning and
 immunolabeling of larvae
 (protocol), 139–145,
 141f–142f
- Peripheral nerve crush in *Drosophila* larvae
 (protocol), 41–50, 43f, 45f
 discussion, 47–48
 imaging and analysis (nerves/axons),
 44–46, 45f
 imaging and analysis (neuromuscular
 junctions), 45f, 46
 injury and dissection, 42–44, 43f
 materials, 41–42
 method, 42–46, 43f
 recipes, 48–49
 troubleshooting, 46–47
- Peripheral nervous system (PNS)
 dissection and immunolabeling of larvae
 (protocol), 131–138, 133f
 glial cells, 121–155

- Peristalsis, 790–791
 PHASE, 866
 Phase locking value (PLV), 506
 PHD (presynaptic homeostatic depression), 402f, 404, 406
 Phenol–chloroform–isoamyl alcohol
 method of DNA extraction, 305
 Pheromones
 aggression, 848–849
 reproductive behavior, 706–708, 723, 736
 Philanthotoxin-433 (PhTx), 404, 405t, 410–412, 414
 Philanthotoxin-433 (PhTx) stock solution (recipe), 415, 421, 427
 Phosphate-buffered saline (PBS) (recipe), 49, 56, 82, 91, 102, 113, 137, 145, 155, 177, 184, 191, 197, 670
 Phosphate-buffered saline (PBS; 1×; pH 7) (recipe), 24, 30
 Phosphate-buffered saline with 0.1% (v/v) Triton X-100 (PBS-T) (recipe), 137, 145, 155
 Phosphate-buffered Triton (PBST; 0.1%) (recipe), 177, 184, 191, 197
 Phospholipase C (PLC), 614
 Photobleaching, 424–425, 511, 570–573, 578–581, 600–602, 608–609
 Photolithography, creating channel molds using, 223
 Photoreceptors
 calcium imaging of neural activity in photoreceptors, 509–530
 functional imaging (protocol), 521–526, 522f, 524f
 overview, 509–510
 PHP. *See* Presynaptic homeostatic potentiation
 PhTx (philanthotoxin), 404, 405t, 410–412, 414
 Pigment-dispersing factor (Pdf), 432
 Pinch assay, 42, 47
 PINK1 (PTEN-induced kinase-1), 634–635
 Pipettes for patch-clamping neurons
 homemade pipette-filling devices, 438
 polishing pipettes, 437–438
 protocol, 435–441
 pulling pipettes, 436–437, 437t
 Sutter puller, 435–440, 436t–437t
 PLA. *See* Proximity ligation assay
 Plasticity
 learning-induced, 583–611
 nociceptive, 765–766
 PMSF (phenylmethylsulfonyl fluoride) (recipe), 308, 314–315
 PNS. *See* Peripheral nervous system
 Polyandry, 708
 Polydimethylsiloxane (PDMS) larva chip, 51–55, 53f–54f
 Polyvystin cation channel, 69
 Positional preference analysis using multibeam activity monitors (protocol), 890–895
 discussion, 894
 materials, 890–891
 method, 891–893, 891f–894f
 recipes, 894–895
 Postmating receptivity, 739, 741f
 Postprandial inhibition, 709
 Presynaptic calcium influx at larval neuromuscular junction (protocol), 422–427, 425f
 data analysis, 426
 dye loading for baseline calcium imaging, 423–424
 dye loading for calcium imaging during PHP, 424
 imaging by confocal microscopy, 424–426, 425f
 materials, 422–423
 method, 423–426, 425f
 recipes, 426–427
 troubleshooting, 426
 Presynaptic homeostatic depression (PHD), 402f, 404, 406
 Presynaptic homeostatic potentiation (PHP)
 elicitation at larval NMJ (protocol), 410–416
 factors needed for long-term maintenance, 406t
 glutamate receptor loss of function, 403–404, 404f
 homeostatic mutants that affect, 407
 models, 403
 overview, 402–403, 402f
 repressors of function, 407t
 through pharmacology, 404, 405t
 Presynaptic homeostatic potentiation (PHP) elicitation at larval NMJ (protocol), 410–416
 cleaning and washing larval preparation, 412–413
 data analysis, 414
 larval dissection, 412
 materials, 410–411
 method, 411–414
 rapid induction of PHP, 412
 recipes, 415
 recording evoked postsynaptic potential, 413–414
 recording membrane potentials and mEPSPs, 413
 troubleshooting, 414–415
 Prime editing gRNAs (pegRNAs), 325
 Probe hybridization buffer (recipe), 259
 Probe wash buffer (recipe), 259
 Proboscis-extension response (PER), 691, 692, 696–697, 696f, 701f, 702–703
 Proprioception, 68–69, 74, 210
 Protease pipette, 436–437, 437t, 439–440, 445, 451, 458, 459f, 462, 466
 Protease pipettes (recipe), 466
 Protease treatment for removal of superficial glia, 456–459
 Protein localization, in perineurial glia studies, 125–126
 Protein–protein interactions, detecting, 127, 146
 Protein synthesis-dependent long-term memory (LTM), 812–813
 Protospacer-adjacent motif (PAM), 324, 324f, 330–334, 339
 Proximity ligation assay (PLA)
 disadvantages, 128
 for fillets of larvae (protocol), 146–155
 overview, 127–128
 to visualize colocalization of proteins at larval NMJ (protocol), 193–197, 195f
 Proximity ligation assay (PLA) for fillets of larvae (protocol), 146–155
 amplification-detection, 151
 controls required, 148–149
 dissection and fixation of larvae, 149–150
 example images, 153f
 final wash and preparation for imaging, 151–152
 flowchart, 149f
 imaging, 152
 immunolabeling of experimental and control samples, 150
 ligation, 151
 materials, 146–148
 method, 148–152, 149f
 probes, 150–151
 recipes, 154–155
 troubleshooting, 152–154
 Proximity ligation assay use to visualize colocalization of proteins at larval NMJ (protocol), 193–197, 195f
 discussion, 196
 materials, 193–194
 method, 194–196, 195f
 recipes, 196–197
 Proximity ligation assay wash buffer A (recipe), 155
 Proximity ligation assay wash buffer B (recipe), 155
 PTEN-induced kinase-1 (PINK1), 634–635
 Pupa
 collection and staging, 97–98, 97f

Index

- Pupa (*Continued*)
 culture of larval and pupal *Drosophila*
 dendritic arborization
 neurons (protocol), 105–114,
 106f, 108f, 110f
 in *Drosophila* life cycle, 2–3
 isolation, 857
 mounting of embryos, larvae, and
 pupae for live *Drosophila*
 dendritic arborization neuron
 imaging (protocol), 92–104
 optic lobe, 267, 267f
 Pupal brain
 dissection, 272–273, 273f
 mounting sections, 276–277
 PySolo, 866
- Q**
- Quantal units of transmitter release, 359,
 360f
- Quantitative analysis of photoreceptor
 intensity-response function
 in visual neurons (protocol),
 527–530, 528f
 data processing and analysis using
 MATLAB, 529
 discussion, 529–530, 530f
 fitting data with Naka–Rushton
 function, 529
 image registration, 527–528
 materials, 527
 method, 527–529, 528f
- Quantitative PCR (qPCR)
 after chromatin immunoprecipitation
 (ChIP), 297–298, 306,
 317–321, 319f
 of antennal and brain sample, 317–321,
 319f
- Quantitative PCR after ChIP of antennal
 and brain samples
 (protocol), 317–321, 319f
 data analysis, 320
 materials, 317–318
 method, 318–320, 319f
 primer amplification efficiency
 measurement, 318–320, 319f
 primer design, 318
- Quench cross-linking buffer (recipe), 308
- R**
- Rac1, 72
 Radioactive safety procedures, 910
 RalA, 124
 Raspberry Pi Virtual Reality (PiVR) system
 introduction to, 789–790
 larval chemotaxis with odor gradients
 (protocol), 793, 795–809,
 802f–806f
 setup, 792f
 Raspberry Pi Virtual Reality (PiVR) system
 study of larval chemotaxis
 with odor gradients
 (protocol), 795–809
 agarose dishes, preparation of, 796–797,
 797f
 camera, 804–805
 choosing appropriate larval stage,
 801–802
 conducting a real odor experiment,
 798–799
 conducting a virtual chemotaxis
 experiment, 799–800
 discussion, 801–807
 distance to source, 806, 806f
 effect of resolution on tracking noise
 and filtering, 804f
 experimental conditions, appropriate,
 803
 graphical user interface, 805–806,
 805f
 head–tail classification, 805–806, 805f
 larvae preparation, 797–798, 798f
 materials, 795–796
 method, 796–800
 odor preparation, 798
 Overview of tracking file, 803, 803f
 PiVR setup, 803
 recipes, 807–808
 running analysis scripts, 807
 speed of larval movement, calculating,
 806
 sucrose washing solution, 803
 technical considerations, general,
 801–807
 troubleshooting, 800
 unlocking potential of PiVR setup, 801
- RC (resistor–capacitor) circuit, 349,
 351b–352b, 368
- Readily releasable synaptic vesicle pool
 (RRP) at larval NMJ
 (protocol), 417–421, 420f
 data analysis, 419
 materials, 417–418
 method, 418–419, 420f
 recipes, 421
 recording EPSCs during high-
 frequency stimulus trains,
 419, 420f
 recording membrane potentials,
 418–419
 troubleshooting, 420–421
- reaper, 198
 Reciprocal appetitive training, 836
 Reciprocal training
 aversive, 831
 aversive odor–high salt learning,
 672–673
 aversive olfactory–taste learning and
 memory in larvae (protocol),
 683–684
- Recording. *See also* Electrophysiological
 recording
 membrane potential, 375–377
 neuronal activities, 343–345, 344t
 synaptic potentials, 377–379
 whole-brain electrophysiology during
 sleep and wake, 467–508
- Recording from larval body wall muscles
 (protocol), 374–382,
 377f–378f
 calcium-dependent short-term synaptic
 plasticity, 379, 380f, 381
 data analysis, 380
 discussion, 381
 dissection third-instar larvae, 375
 electrode cleanup, 379–380
 materials, 374–375
 method, 375–380, 377f–378f
 recipe, 382
 recording membrane potentials,
 375–377
 recording synaptic potentials, 377–379
 troubleshooting, 380–381
- Recording neuronal activities
 current vs. voltage clamp, 344t, 345
 direct, 343–345
 extracellular, 343–345, 344t
 intracellular, 344t, 345
- Reflexive feeding response (protocol), 692,
 694–698, 695f–696f
 analysis of proboscis-extension reflex
 data, 697
 experiment preparation, 695–696, 695f
 fly preparation, 696–697, 696f
 materials, 694–695
 measurement, 697
 method, 695–697, 695f–696f
- Regeneration of axonal injury, 32–33,
 47–48
- Relative permeability, 358
 Remating, 739–740
- Reproductive behaviors, 705–744
 acoustic communication during
 (protocol), 730–735, 731f
 balancing courtship motivation with
 other drives, 709
 courtship behaviors, 705–710, 706f
 courtship conditioning/suppression assays
 (protocol), 723–729, 726f
 female postmating behaviors, 706f,
 707–708, 736–744, 741f
 methods for measuring, 709–710
 modulation of male behaviors, 708–709
 single-pair courtship and competitive
 assays (protocol), 714–722,
 715f

- Resistance (R or W), 350b
Resistor–capacitor (RC) circuit, 349, 351b–352b, 368
Resolution, 139, 570
Resting membrane potential, 350–359, 354f
Rethomics, 866
Reversal potential, 357–359
RGECO, 511, 513
RhythmicAlly, 866
Richet, Charles, 158
Rinaldini solution (recipe), 113
RN-Gal4 driver, 432, 458
Robo, 5–10, 8f
robo, 5–7
Rolling behavior, 763–765, 772–773, 775, 778, 781, 784–785
Rtivity, 866
Runs, 790–791. *See also* crawling
Rutabaga, 159
Ryanodine receptors, 613
- S**
- S2 cell growth medium, 191
Safety
 biological safety procedures, 911
 disposal of laboratory waste, 909–910
 general cautions, 908–909
 hazardous chemicals, properties of, 911–912
 information resources, 907
 Material Safety Data Sheets (MSDSs), 907
 radioactive safety procedures, 910
SARM1, 32
Scalloped, 71
SCAMP (Sleep and Circadian Analysis MATLAB Program), 890–891, 893
 analysis of sleep and circadian rhythms from DAM data (protocol), 896–905, 897f–904f
 downsides, 866–867
schizo, 6
Scientific complementary metal oxide semiconductor (sCMOS) camera, 571
Selective membrane permeability, 353
Self-avoidance, dendrite, 72
Semaphorin 2a, 159
Sensilla
 chemosensory coding in single sensilla, 531–568
 olfactory, 532–534, 553f
Sensorimotor circuit assembly, 205–260
 Drosophila as a study model, 206
 Drosophila larval body and movement, 206–207, 207f
 fluorescent in situ hybridization chain reaction for RNA in embryonic and larval central nervous system (protocol), 246–260, 248f, 251f–252f, 255f
 imaging neural activity in intact, semirestrained larvae (protocol), 227–235, 230f, 232f
 larval behavior in agarose channels (protocol), 214–226, 218f
 larval neurons, 208–209
 overview, 205–212
 single-neuron labeling using multicolor FLP-out (protocol), 236–245, 239f, 242f
Sensory neurons, larval, 208–209
Sequential assembly, 210
Shannon entropy, 506
Sharp electrophysiology, 430–431, 431f
Sharpened forceps (recipe), 446, 453
Shibire (tool), 850, 872
shibire gene, 361
Shiny, 866
Shipping biological material, 911
Shock, in classical conditioning, 812–813, 816, 830–832, 839–840, 842
Shock acuity assay, 840
Short neuropeptide (sNPF) neurons, 869–870
Short terminal branchlets (STBs), 68, 70–72
Shutter, electronically controlled, 571
Sibling matching, 210
Silver wire, chlorination of, 457b
Simultaneous photobleaching and imaging (SPAIM), 572
Single-neuron labeling using multicolor FLP-out (protocol), 236–245, 239f, 242f
 discussion, 243
 heat shocking embryos, 238–239
 image analysis, 241–242, 242f
 larval CNS dissection and fixation, 239–240
 larval CNS immunofluorescence, 240
 larval CNS mounting, 241
 materials, 236–238, 239f
 method, 238–242, 242f
 recipes, 244–245
 troubleshooting, 242–243
Single-pair courtship and competitive assays (protocol), 714–722, 715f
 cleaning courtship chambers, 717
 collection and rearing of flies, 716
 data analysis, 717–719
 discussion, 720
 materials, 714–716, 715f
 method, 716–719
 testing, 717
 troubleshooting, 719–720
Sleep
 background on research, 467–470
 whole-brain calcium imaging during sleep and wake (protocol), 489–508
 whole-brain electrophysiology during sleep and wake (protocol), 473–488, 476f–477f, 480f–481f, 485f
 as whole-brain phenomenon, 469–470
Sleep, analysis of, 863–905
 analysis of DAM data using SCAMP (protocol), 896–905
 background, 863–864
 beam breaks, 864–867
 future directions, 874–875
 neural stimulation during DAM-based studies (protocol), 879–890
 optogenetic approaches, 868–872, 869t
 positional preference analysis using multibeam activity monitors (protocol), 890–895
 thermogenetic approaches, 869t, 872–874
 video-based tracking, 867–868
Sleep deprivation, 468, 493–494
Sliding chamber assay, 858f, 858t, 859
Slit, 5–8, 8f
Small lateral ventral neurons (sLNs), 432
SNAP-25, 359–361
sNPF (short neuropeptide) neurons, 869–870
Society for Development Biology, 2
Sodium chloride–sodium citrate buffer (SSC; 20×) (recipe), 259
Song, courtship, 706–707, 720, 730–735, 731f
Sonication of chromatin, 303, 306
SOP-FLP, 79–81
Sound stimulus, for studying neural activity, 229–230
Spaghetti monster GFPs, 243
SPAIM (simultaneous photobleaching and imaging), 572
Spastin, 71
“Spring” model, 358–359, 358f
SSCT (5×) (recipe), 260
Startle response, 760
STBs (short terminal branchlets), 68, 70–72
Stem cells. *See* Neural stem cells
Sterile handling, 911
Stimulator, 364
Subperineurial glia, 124–125
Subsynaptic reticulum, 160

Index

- Sucrose rewards, 812–813, 832, 836
 Sugar acuity, measuring, 840
 Sugar paper, 832–833, 833f, 842
 Superficial glia, protease treatment for removal of, 456–459
 Support vector machines (SVMs), 486
 Sutter horizontal puller, 435–440, 436t–437t
 Sylgard, for channel mold production, 216, 223
 Sylgard 184 Silicone Elastomer Kit, 445
 Sylgard-coated coverslips (recipe), 453
 Sylgard-coated glass petri dishes (recipe), 138
 Sylgard-coated Petri dishes (recipe), 447, 454
 Sylgard plates, 177
 Synapses, glutamatergic, 158
 Synaptic electrophysiology of neuromuscular junction, 349–399
 background, 349–350
 electrophysiological recording from a “model” cell (protocol), 368–373, 369f–371f
 electrophysiology equipment, 362–365, 362t, 363f, 365f
 fabrication of microelectrodes, suction electrodes, and focal electrodes for electrophysiological recording, 394–399, 396f, 398f
 focal recording of synaptic currents from single boutons at larval NMJ (protocol), 390–393
 recording from larval body wall muscles (protocol), 374–382, 377f–378f
 resting membrane potential, 350–359, 354f
 synaptic transmission, 359–365, 360f
 voltage-clamp analysis of synaptic transmission (protocol), 384–389, 387f
 Synaptic homeostats, 401–402
 Synaptic potentials, recording, 377–379
 Synaptic transmission
 electrophysiology equipment, 362–365, 362t, 363f, 365f
 history of neurogenic and electrophysiological studies, 360–361
 molecular nature, 359
 overview, 359–361, 360f
 using larval neuromuscular junction to study, 361–365
 Synaptic vesicle cycling, imaging of, 346
 Synapto-pHluorins, 346, 588
 Synaptosomal-associated protein 25 (SNAP-25), 359–361
 Synaptotagmin, 359, 361
 Syntaxin, 359–361

T
 Tachykinin, 851
 TAE buffer (50×) (recipe), 308
 Tagged extracellular domains (ECDs), 186
 Tagged proteins, in perineurial glia studies, 125–126
 Taste discrimination, 693, 703
 Taste hairs, 533–534, 533f
 Taste learning
 appetitive odor–taste learning, 674
 aversive odor–taste learning, 673–674, 678–689
 Taste memory, aversive, 678–689, 692–693, 703
 Taste memory, measurement of (protocol), 699–704
 analysis of aversive taste memory data, 703
 experiment preparation, 700
 fly preparation, 701, 701f
 materials, 699–700
 measurement, 702–703
 method, 700–703, 701f
 taste discrimination, 703
 Taste neurons
 chemosensory coding principles, 539–540
 electrophysiological recordings from chemosensory sensilla, 537–538
 receptors, 535–536
 recording from taste sensilla (protocol), 558–568, 561f–562f, 566f
 Taste pegs, 534, 534f
 Taste processing, 691–704
 aversive taste memory, 692–693
 introduction, 691–692
 proboscis-extension response (PER), 691, 692, 696–697, 696f, 701f, 702–703
 reflexive feeding response (protocol), 692, 694–698, 695f–696f
 taste memory, measurement of (protocol), 699–704
 Taste sensilla, recording from (protocol), 558–568, 561f–562f, 566f
 data analysis, 565–566, 566f
 discussion, 567
 fly preparation, 562–564, 562f
 labellar sensilla, recording from, 562–563, 562f
 leg sensilla, recording from, 563–564
 materials, 558–560
 method, 560–566, 561f–562f, 566f
 performing recordings, 564–565
 recipe, 567
 recording electrodes and micropipette washer, 561–562
 recording rig assembly, 560–561, 561f
 tastant preparation, 560
 troubleshooting, 566
 Taste sensilla anatomy, 532–534, 533f
 TE buffer (10×, pH 8.0) (recipe), 308, 315
 Temporal cohort, 210–211
 Temporal matching, 209–210
 Temporal patterning, and neural stem cells, 283f
 Tenurin-m, 70
 Territory, 847–848, 850, 862
 Tetanus toxin, 765
 Tethering
 process close-up, 477f
 setup and hardware, 476f
 for two-photon imaging, 494–497, 494f
 for whole-brain recordings, 476–478, 476f–477f
 TEVC (two-electrode voltage-clamp) method, 384–389, 387f
 Thermal nociception, 764
 Thermogenetic approaches to sleep study, 869t, 872–874
 benefits, 872–873
 limitations, 873
 methodological considerations, 873–874
 neural stimulation during DAM-based studies of sleep and circadian rhythms (protocol), 879, 883, 886, 887f
 3D printing, of agarose channel molds, 216, 223
 Three-photon technology, 589
tipsy, 747
 T-maze apparatus, 811–813, 816–842
 assembling, 822–826
 aversive and appetitive multisensory conditioning, 836–839
 for aversive olfactory conditioning, 828–832
 aversive training tube assembly, 824–825, 824f
 bench setup preparation, 826
 collection tube preparation, 826–827, 827f
 elevator assembly, 822–823, 822f
 fly collection device, cleaning and assembling of, 827–828, 827f
 hardware parts, 819t
 odor delivery vial assembly, 825–826, 825f
 preparing parts for assembly, 821–822, 821f

- printed parts, 820t, 821, 821f
 setup, 820f, 826–827, 826f
 setup for appetitive olfactory
 conditioning procedure, 833,
 834f
 setup for aversive and appetitive
 multisensory conditioning,
 837, 838f
 setup for aversive olfactory conditioning
 procedure, 829–830, 829f
 side walls and base assembly, 823, 823f
 standard tube assembly, 823–824, 824f
 switch box assembly, 825
 Tracking navigation behavior in odor
 gradients, 789–809, 790f
 Training. *See* Classical conditioning
 Transcriptional genetically encoded
 calcium indicators (GECIs),
 613–631
 background, 613–615
 CaLexA, 615–618, 624–631
 TRIC, 618–620, 619f, 624–631
 Transgenes for labeling neural subsets in
 Drosophila, 9t
 Transient receptor potential (TRP), 69, 512
 Transient receptor potential-like (TRPL
 channels), 512
 Transient receptor potential subfamily C
 (TRPC) channels, 613
 Transmembrane channel-like protein, 69
 TRIC
 design principles, 618, 619f
 example applications, 620
 fly lines available, 625t
 imaging signals in dissected fly CNS
 (protocol), 624–631
 parameters to be considered when
 using, 618–620
 TriKinetics, Inc., 865–867, 881–882, 886,
 890, 893
 Tris-Acetate-EDTA (TAE) buffer
 (50×) (recipe), 341
 Tris-HCl (recipe), 308, 315
 TRP (transient receptor potential), 69, 512
 TrpA1, 69, 850, 872
 Tungsten electrode sharpener, assembly
 and use of, 547–548, 548f
 Tungsten tools, generating ideal sharpened,
 170–172, 171f
 micromanipulator control of electrode
 sharpening, 170, 171f
 setup of tungsten wire-sharpening rig,
 170
 sharpened tungsten embryo tool,
 171–172
 tungsten pin sharpening technique, 170
 Turtle, 72
 Two-electrode voltage-clamp (TEVC)
 method, 384–389, 387f
 Two-odor reciprocal aversive odor–high
 salt learning, 672–673
 Two-photon microscopy
 calcium imaging in visual neurons,
 511–512
 dissection of caudal cuticle for, 496f
 dissection of dorsal cuticle for, 497f
 functional imaging from
 photoreceptors (protocol),
 521–526, 522f
 imaging of learning-induced plasticity,
 589
 tethering for imaging, 494–497, 494f
 for whole-brain calcium imaging, 470f,
 491–493, 491f
 U
 Unpaired 2 (upd2), 660
 V
 VAMP (vesicle-associated membrane
 protein), 359–361
 vasa-Cas9, 329
 Vectashield Antifade Mounting Medium,
 147, 181
 Vein, 124
 Ventral nerve cord (VNC)
 anatomy, 208f
 dissection, 28–30, 28f
 neuromuscular circuit development,
 158–160
 sleep regulation, 864
 Ventral nerve cord dissection and
 microscopy of *Drosophila*
 embryos (protocol), 25–30
 dissecting ventral nerve cords, 28–30,
 28f
 materials, 25–26
 method, 26–30
 recipes, 30
 sorting and selecting individual
 embryos for dissection, 27–28
 tungsten dissection needles, preparing
 and sharpening, 26–27, 27f
 tungsten needle sharpener, assembling,
 26
 Vesicle-associated membrane protein
 (VAMP), 359–361
 Vibration isolation table, 362
 Video-based tracking of sleep, 867–868
 Video monitor system, 365
 Virgator lines, 720
 Visual system
 calcium imaging of neural activity in
 photoreceptors, 509–530
 optic lobe development, 261–278
 overview, 261–262
 VNC. *See* Ventral nerve cord
 Voltage (V), 350b
 Voltage-clamp analysis of synaptic
 transmission at larval
 neuromuscular junction
 (protocol), 384–389, 387f
 materials, 384–385
 method, 385–387, 387f
 recipe, 388
 troubleshooting, 387–388
 Voltage clamp recording, 344t, 345
 Voltage-gated channels, 356–357, 613
 von Frey filament, 770, 771f, 772–773
 W
 Wallerian degeneration, 32, 35, 37f, 38, 41,
 47, 61
 Wallerian degeneration and clearance of
 olfactory receptor neuron
 axons following *Drosophila*
 antennal transection
 (protocol), 35–40, 37f
 discussion, 38
 materials, 35–36
 method, 36–38, 37f
 recipe, 39
 Wash buffer I for ChIP (low-salt) (recipe),
 315
 Wash buffer II for ChIP (high-salt)
 (recipe), 316
 Wash buffer III for ChIP (recipe), 316
 Waste disposal
 basic rules, 909–910
 biological waste, 911
 general cautions, 908–909
 radioactive waste, 910
 WAVE, 72
 white, 720
 Whole-brain calcium imaging
 background, 469
 during sleep and wake (protocol),
 489–508
 two-photon, 470f
 Whole-brain calcium imaging during sleep
 and wake (protocol),
 489–508
 discussion, 505–506
 functional imaging during spontaneous
 sleep, 500
 imaging preparation for functional
 imaging, 499–500
 imaging through back of the head, 495,
 498
 imaging through top of the head,
 495–497, 498–499
 interfering activity and functional
 connectivity during sleep, 504
 materials, 489–493, 490t, 491f–492f

Index

- Whole-brain calcium imaging during sleep and wake (protocol)
 - (*Continued*)
 - method, 493–504, 494f, 496f–497f, 501f
 - optogenetic sleep induction, 500
 - postprocessing of sleep events, 500–501
 - preparation of animals for inducing sleep, 493
 - preprocessing images for functional analysis, 501–504, 501f
 - recipes, 506–507
 - sleep deprivation, 493–494
 - surgical procedures to expose central complex, 497–499
 - tethering for two-photon imaging, 494–497, 494f
 - troubleshooting, 504–505
 - Whole-brain electrophysiology during sleep and wake
 - background, 467–470
 - protocol, 473–488, 476f–477f, 480f–481f, 485f
 - Whole-brain electrophysiology during sleep and wake (protocol), 473–488, 476f–477f, 480f–481f, 485f
 - behavioral analysis, 483–484, 483f
 - calibration, 481–482
 - discussion, 486–487
 - electrode insertion, 480–481, 480f–481f
 - electrophysiology equipment setup, 478–480, 480f
 - fly collection and maintenance, 475
 - integrated analysis, 485
 - LFP analysis, 484–485
 - materials, 473–475
 - method, 475–485, 476f–477f, 480f–481f, 483f
 - recipe, 487
 - stimulus delivery, 482
 - tethering, 476–478, 476f–477f
 - troubleshooting, 485–486
 - Whole-larva cryosectioning and immunolabeling of larvae (protocol), 139–145, 141f–142f
 - blocking and labeling with primary and secondary antibodies, 143
 - cryosectioning, 141–142
 - cryostat preparation, 140
 - hydrophobic barrier preparation, 143
 - immunolabeling of slides, 143
 - materials, 139–140
 - method, 140–144, 141f
 - preparation for imaging, 144
 - recipes, 145
 - sample fixation, 143
 - sample preparation for cryosectioning, 140–141, 141f
 - troubleshooting, 144
 - Wide-field epifluorescence microscope, 569–570
 - Wings
 - clonal imaging of mitochondria in dissected fly wing (protocol), 636, 642–647, 644f, 646f
 - dissection, 644
 - live imaging of mitochondria in intact fly wing (protocol), 636–637, 638–653, 650f
 - Wrapping glia, 122, 123f, 124
- ## Y
- Yeast cornmeal food medium (recipe), 487, 507
 - Yeast paste (recipe), 16, 83, 103, 114, 225, 245, 259
 - Yeast paste for fly aggression assays (recipe), 862