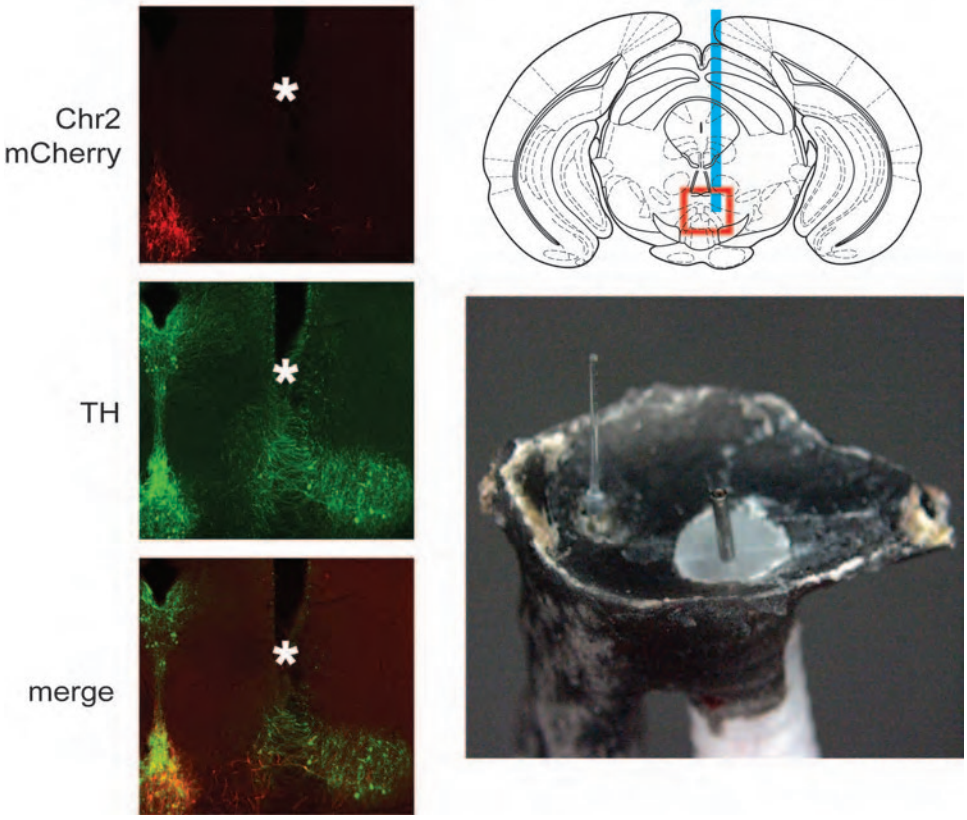
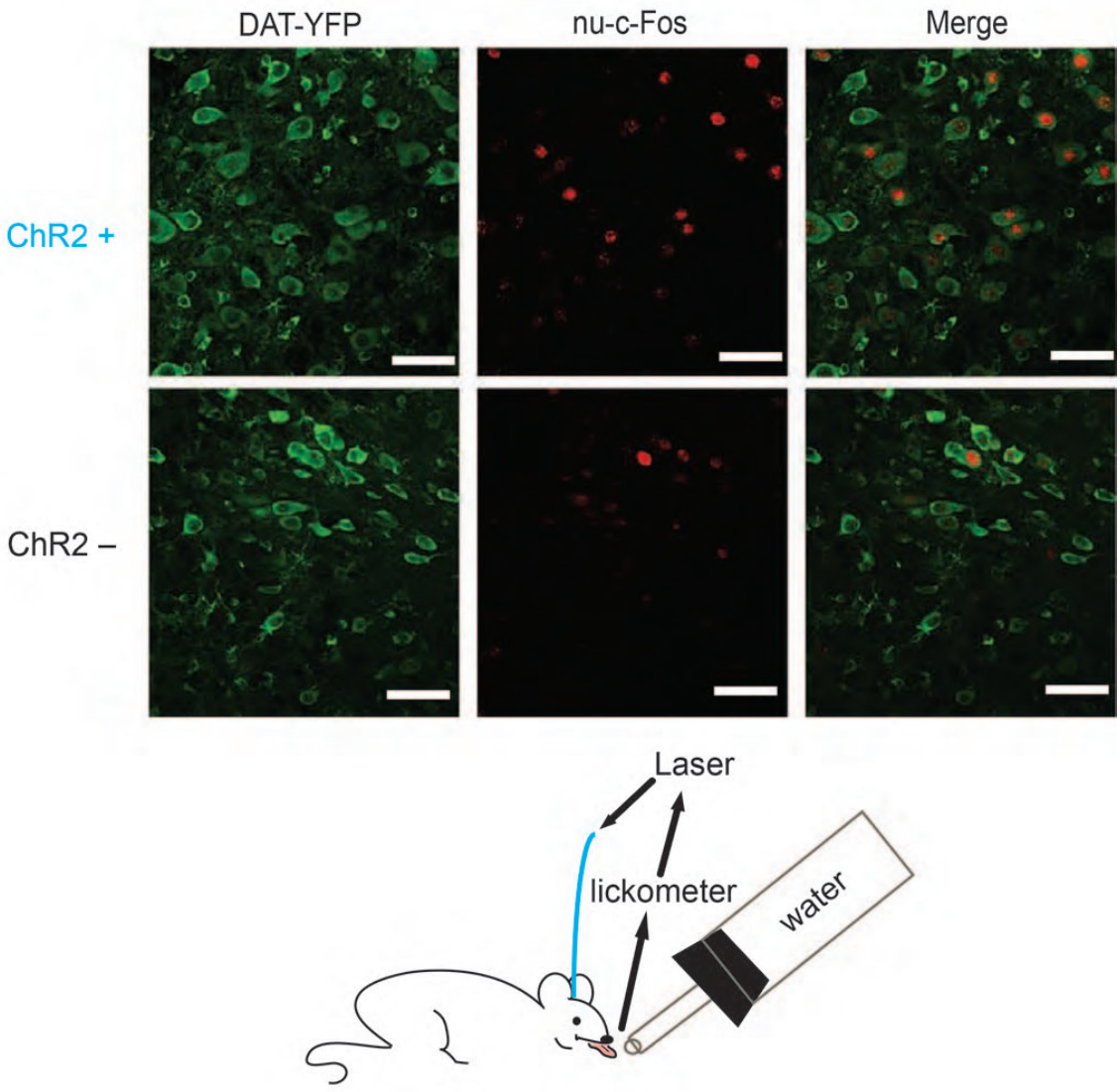
Supplemental Figure-1Friedman



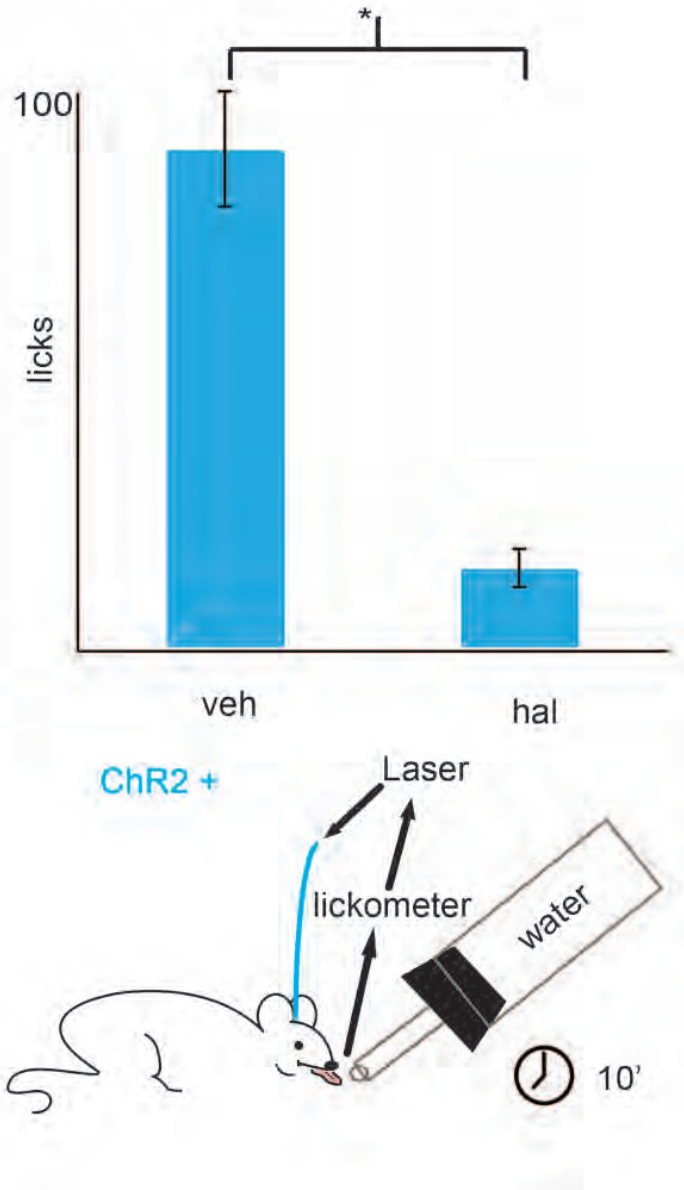
Nature Neuroscience: doi:10.1038/nn.2977

Supplementary Figure-2



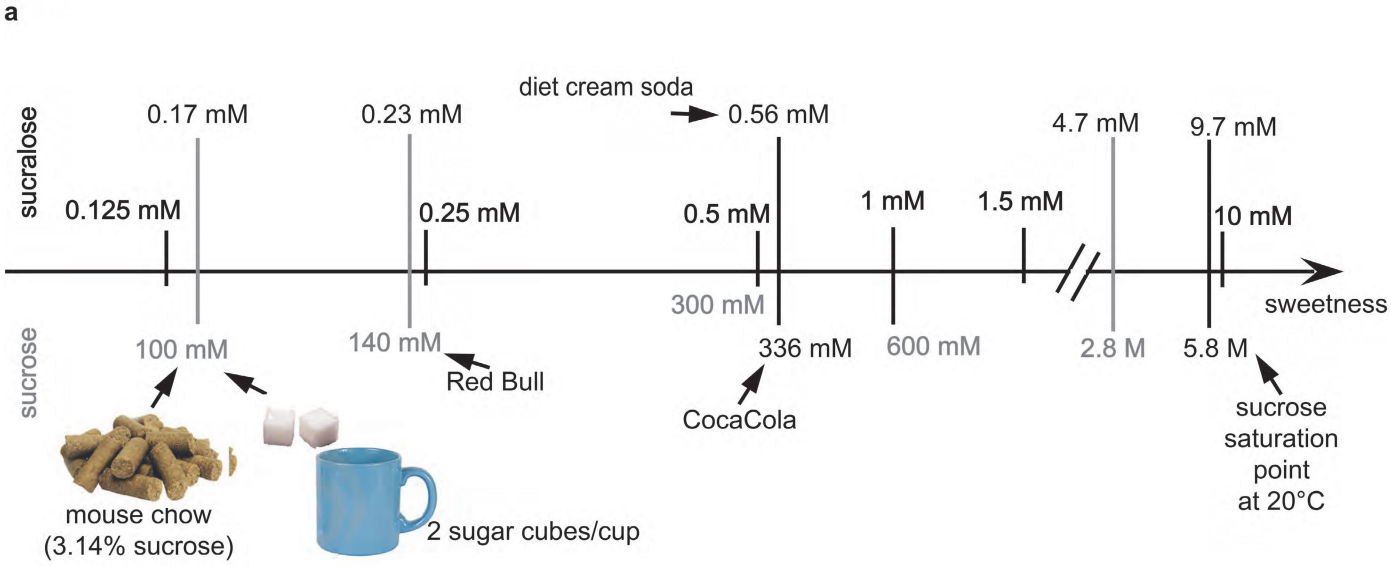
Nature Neuroscience: doi:10.1038/nn.2977

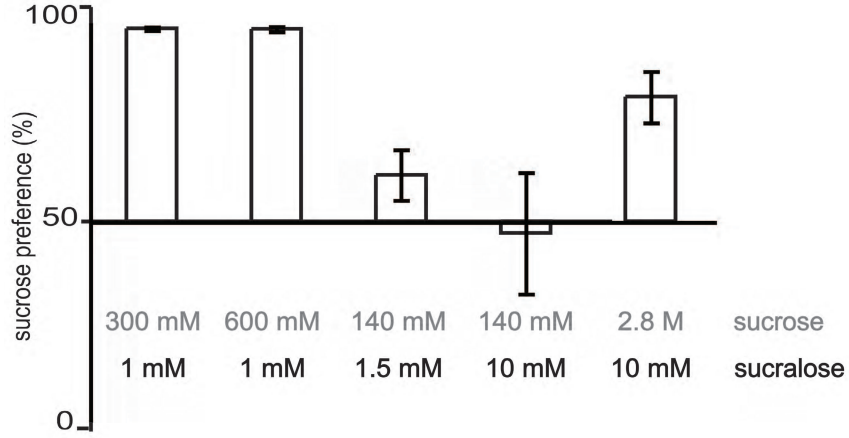
Supplementary Figure-3



Nature Neuroscience: doi:10.1038/nn.2977

Supplementary Figure - 4





Sweet receptor EC_{50} for:

sucrose

T1R2/T1R3: 10 mM<EC_{50}<100 mM, Mice=Human

sucralose

T1R2: 0.01 mM<EC_{50}<0.1 mM, Mice=Human

T1R3: 0.01 mM<EC_{50}<0.1 mM < EC_{50}<1 mM

Human Mice

Supplementary Figure-5

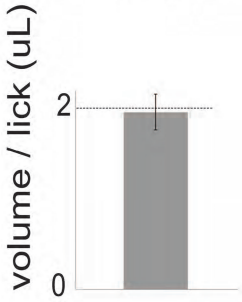
Licks from Supplementary Figure-4b

Sucrose / Sucralose

300 mM	1 mM	600 mM	1 mM	140 mM	1.5 mM	140 mM	10.0 mM	2.8 M	10.0 mM
522	11	454	0	40	57	627	418	116	9
252	1	177	4	24	22	269	224	104	22
671	8	337	6	67	87	70	95	171	57
				123	15	25	269	231	16
				42	29	260	76	46	2
				225	55	259	4	143	63
				116	110	41	182		
				211	63	55	151		

Supplementary Figure-6

a



	mice	humans
mean body weight	20 gr	80 Kg
lick volume	*** 2±0.2ul	* 8mL (1.6 teaspoon)
hipoglycemic rescue	*60 licks @143mM	**1 cup @143mM

*estimate uses mice/human body weight ratio

** [37] (1 tbs=12.86 gr sucrose)

***consistent with [50]

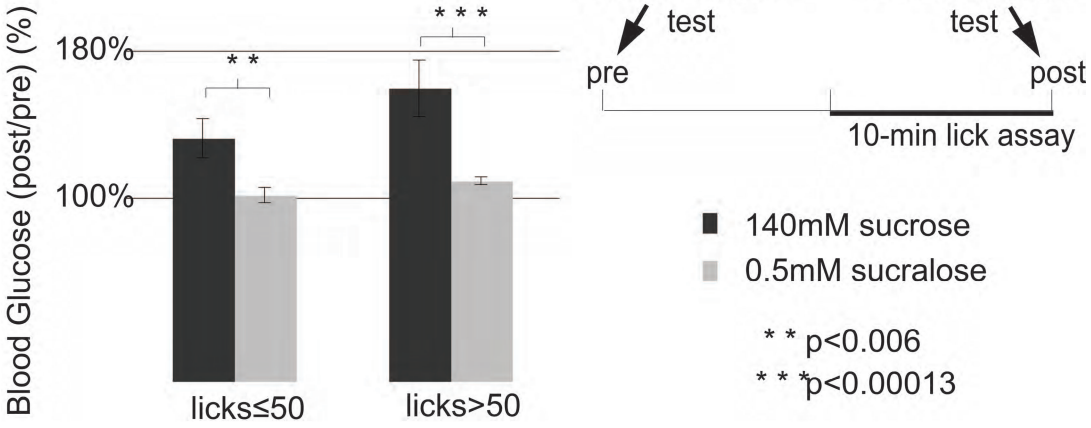
b

* [44.2]

	chow
* sucrose (%)	3.14%
sucrose(mg/gr of chow)	31.4
mean daily chow intake (gr)	4
mean daily sucrose intake (mg)	125.6

sucrose (mM)	100	110	120	130	140
sucrose(mg/uL)	3.423	3.7653	4.1076	4.4499	4.7922
mean licks of ChR2+/run in Fig.3	77	106.8	148.8	76.75	156.333
mean volume/lick(uL)	2	2	2	2	2
mean sucrose/lick	6.846	7.5306	8.2152	8.8998	9.5844
mean sucrose intake(mg)/run in Fig.3	527.142	804.27	1222.4	683.06	1498.4

c



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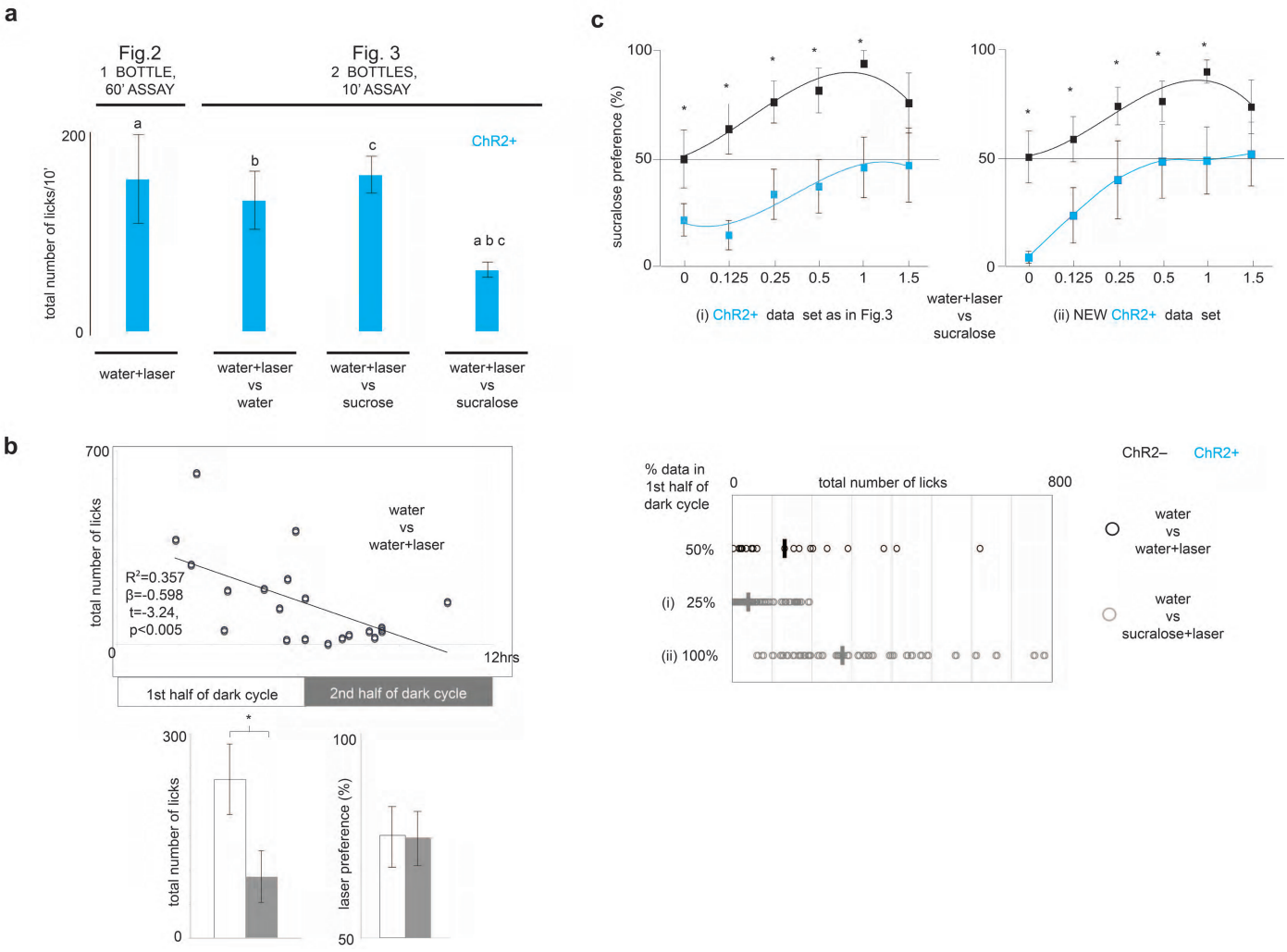
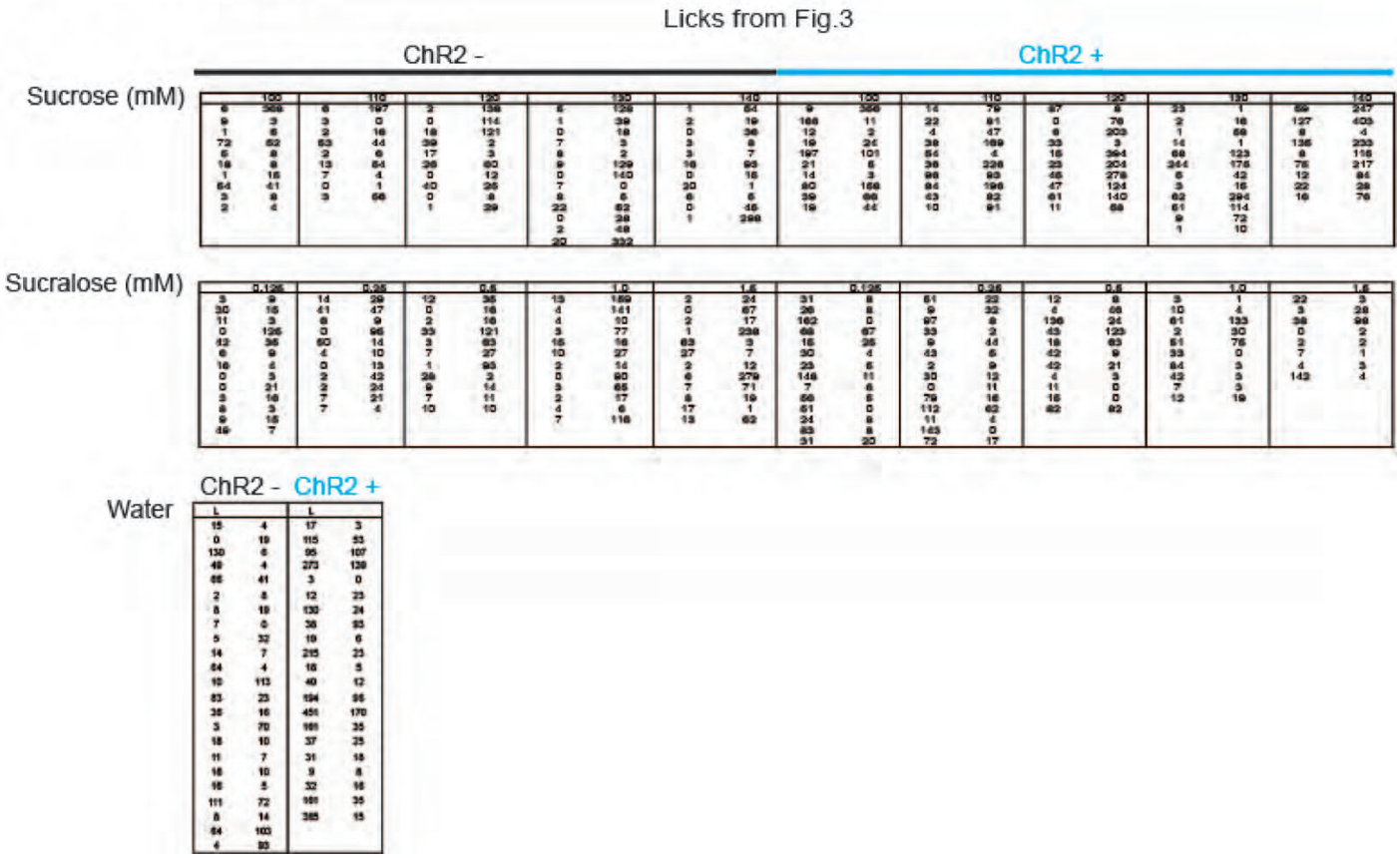
Supplementary Figure - 7



Nature Neuroscience: doi:10.1038/nn.2977

Supplementary Figure 8

Licks from Fig.3



Nature Neuroscience: doi:10.1038/nn.2977

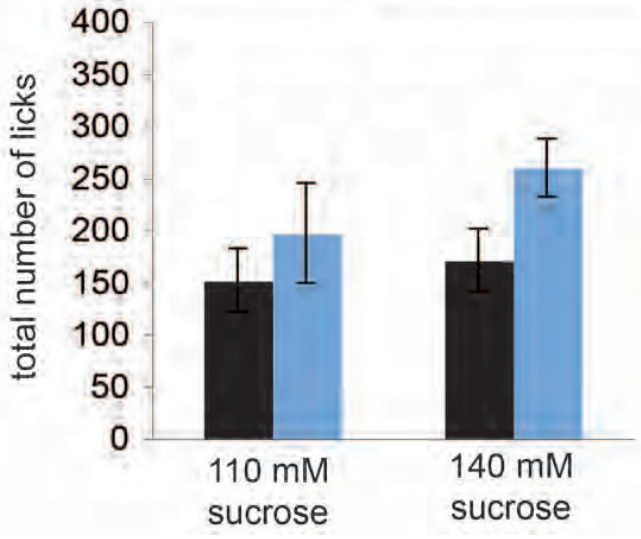
Supplementary Figure-9

Licks from Fig.4

0.5 mM sucralose+laser

vs

	110 mM sucrose		140 mM sucrose	
ChR2 -	13	150	1	163
	9	51	8	92
	35	208	23	171
	48	8	11	114
	19	77	60	212
	84	141		
	40	178		
ChR2 +	15	1	241	148
	315	43	142	20
	5	0	116	44
	342	6	205	8
	247	93	237	3
	85	7	82	122
	198	98	335	4
	196	2	278	18
	81	40	335	0



Nature Neuroscience: doi:10.1038/nn.2977

Supplementary Figure -10

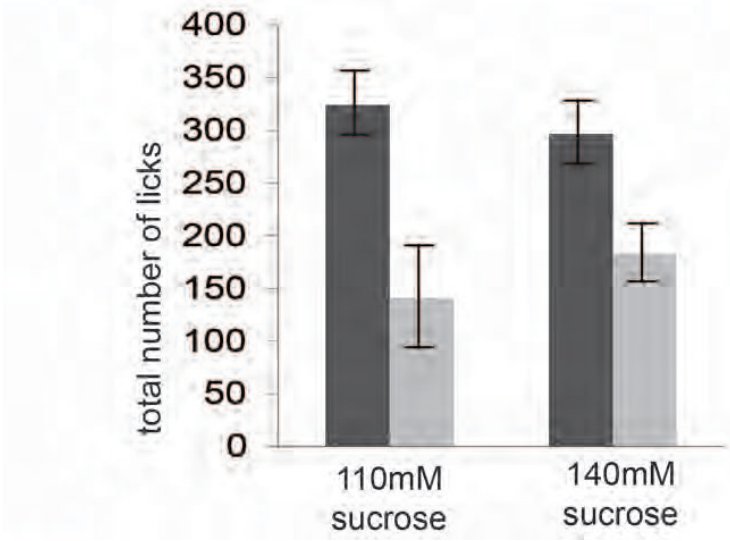
Licks from Fig.5

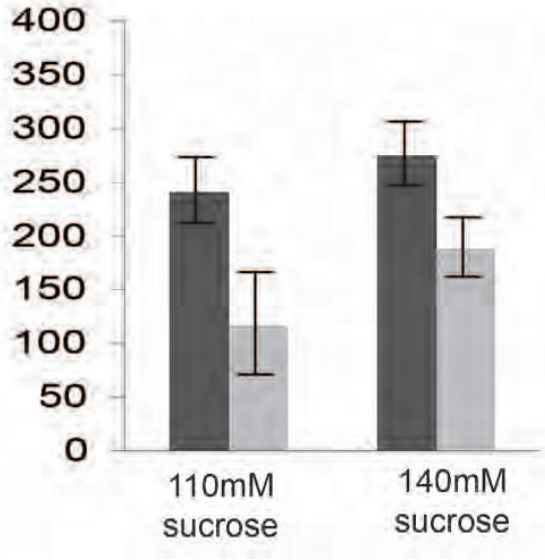
0.5mM sucralose+laser vs

	110mM sucrose		140mM sucrose	
fast +IP veh	43	165	35	115
	1	746	17	700
	12	409	7	183
	7	207	18	249
	59	134	39	211
	7	156	11	194
fast +IP leptin	19	14	24	73
	74	37	210	66
	139	1	286	32
	461	7	155	6
	36	11	19	23
	32	14	20	5

water+laser vs

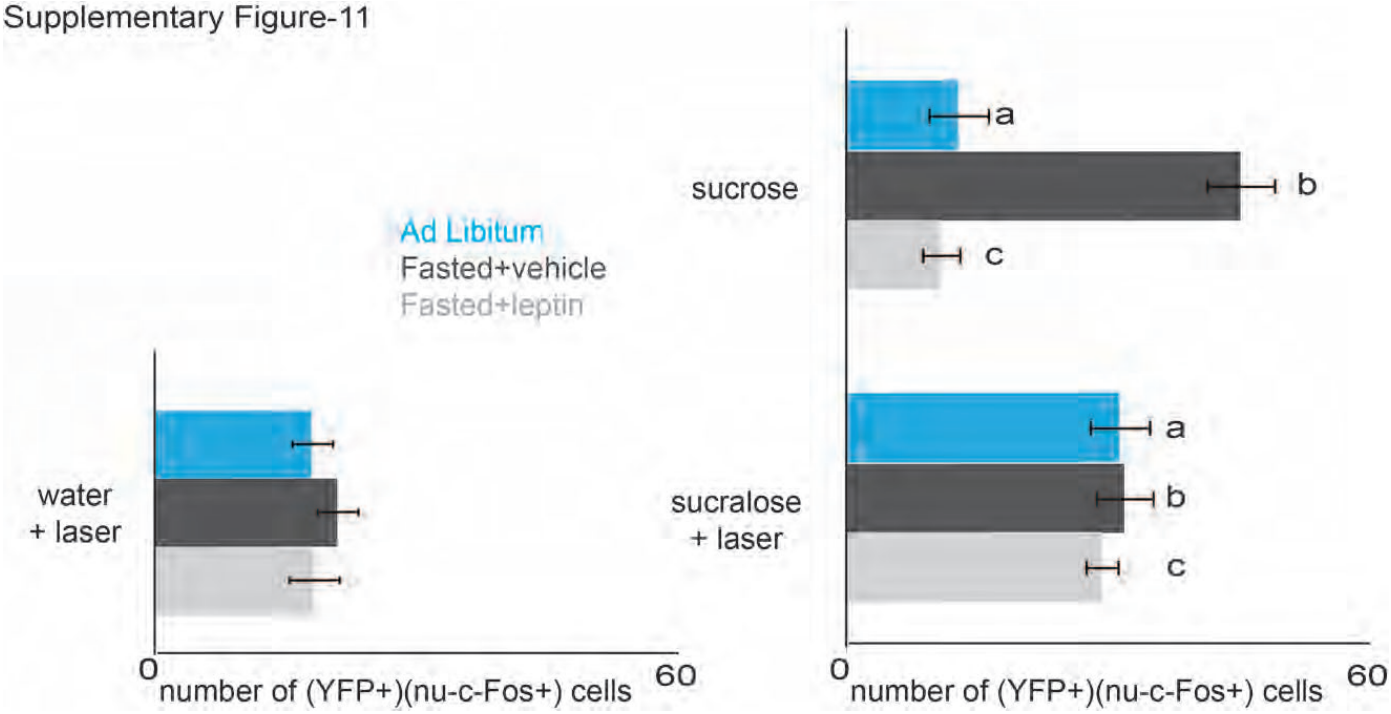
110mM sucrose		140mM sucrose	
64	145	4	156
7	154	95	363
21	183	51	212
236	419	40	218
0	56	9	31
8	153	87	384
14	19	12	73
118	82	56	80
50	44	53	173
21	36	127	50
72	191	29	30
44	12	183	263





Nature Neuroscience: doi:10.1038/nn.2977

Supplementary Figure-11



Nature Neuroscience: doi:10.1038/nn.2977

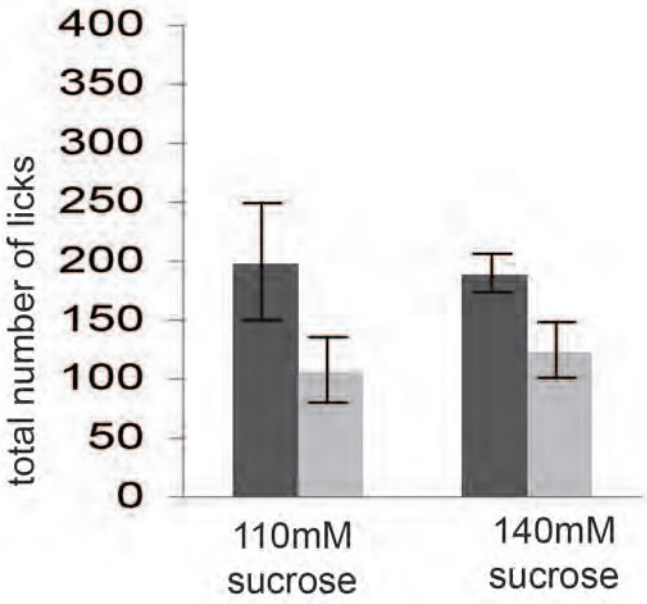
Supplementary Figure-12

Licks from Fig.6

0.5mM sucralose+laser

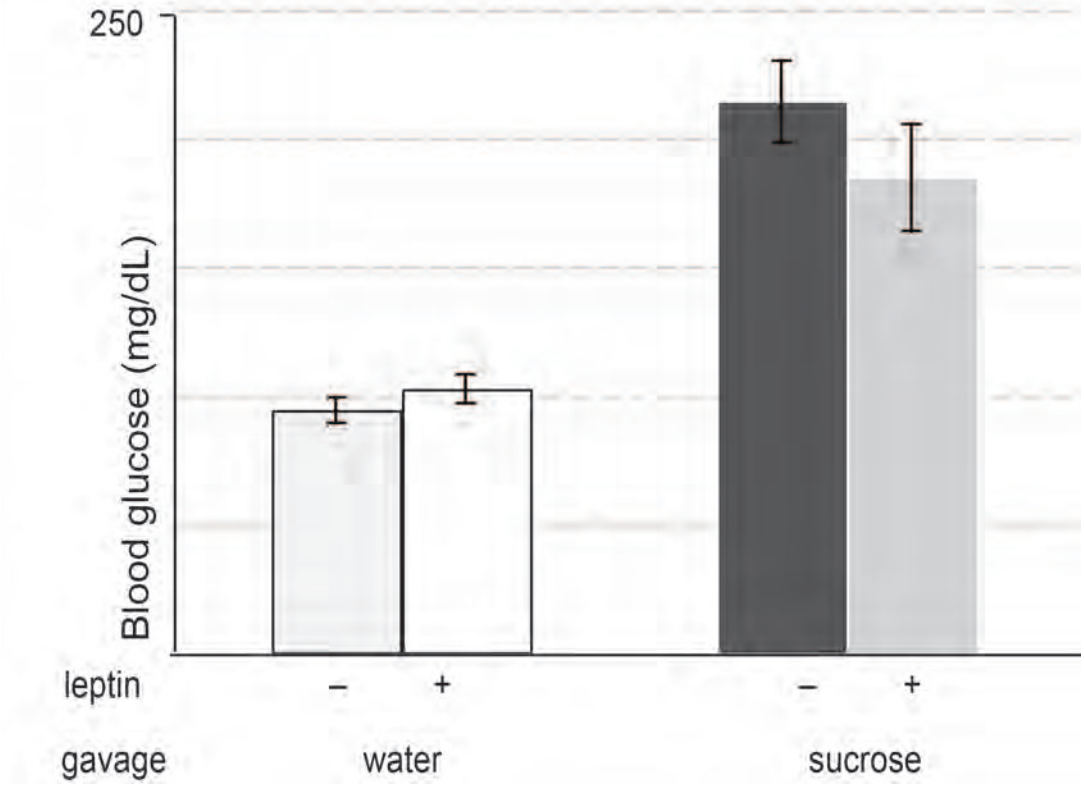
vs

	110mM sucrose		140mM sucrose	
fast +ICV veh	19	53	23	112
	73	142	5	247
	73	225	37	157
	16	185	10	169
	19	26	2	204
	6	349	99	65
fast +ICVleptin	46	14	117	28
	44	29	147	64
	203	4	13	27
	23	3	76	35
	107	58	126	18
	88	19	65	22



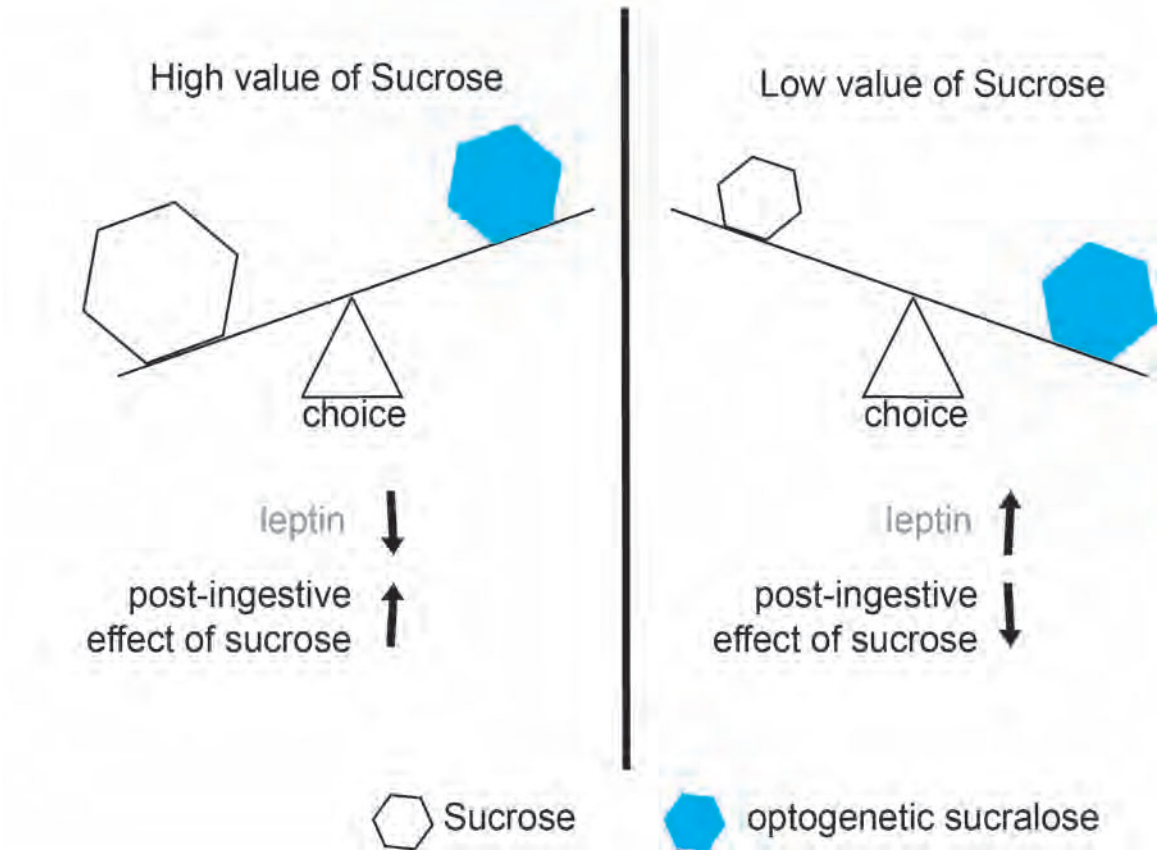
Nature Neuroscience: doi:10.1038/nn.2977

Supplementary Figure -13



Nature Neuroscience: doi:10.1038/nn.2977

Supplementary Figure14



Nature Neuroscience: doi:10.1038/nn.2977

Supplemental Figure Legends

Supplemental Fig.1. Placement of fiber and cannula. Left: Fiber placement is verified by localizing the fiber track relative to ChR2-mCherry (red, top left image) and TH double-positive neurons (green, middle left image); **Right top:** Schematic of fiber placement in the VTA, (Paxinos Atlas). **Right bottom:** Bottom view of a fiber and cannula implant, without screws; Implants endure up to nine months.

Supplemental Fig.2. The optogenetic driven licks induces DA neuron activation. Dat-Cre;Rosa26YFP mice transduced with AAV-DIO-ChR2mCherry, and assayed for 10 minutes as in Fig.2. Co-localization of nuclear cFos (nu-c-Fos) and YFP shows that water+laser activates significantly more DA neurons in ChR2(+)than in ChR2(–) mice ($p<0.0005$), respectively, 14.8±1.9 and 5.2±1 DA neurons per 512^2 $pixel^2$ (n=5).

Supplemental Fig.3. The optogenetic driven-licking is contingent on DA transmission. Blocking dopamine transmission with haloperidol (hal) attenuates the effects of optogenetics. In 10 minutes, ChR2(+)animals injected with hal (ip, 1mg.kg) lick 14.3±3 times, whereas vehicle treated animals lick 97.4±19 times (n=3, significant difference *$p<0.0005$).

Supplemental Fig.4. Sucralose is not preferred to sucrose, at comparable concentration regimes. (a) In order to choose physiological and comparable concentration regimes of sucrose and sucralose, we triangularized information from molecular kinetics (EC_{50}) and information from mouse and human consumption, respectively, mouse chow and popular beverages. In humans, sucralose is 600 times sweeter than sucrose [40]. Popular sugar-sweetened beverages generally have 10% sugar content (~300 mM), but vary on sugar type [39]. RedBull currently has 5.11% sucrose (~140 mM) (see table2 in [39]) among other sugars. In 1983 CocaCola had 11.5% sucrose (page R501, 5^{th} line of

Materials Section in [39]), but it now contains other sugars (see table2 in [39]). Artificially sweetened beverages are generally designed to be as sweet as naturally sweetened drinks, and vary widely in sweetener type. Diet Crush Cream Soda has 82mg/can (0.56 mM) [see www.sucralose.org and http://simple.wikipedia.org/wiki/File:Diet_Crush_Cream_Soda_sweetened_with_Splenda_can.jpg]. Mouse chow contains 3.14% sucrose (5053 PicoLab Rodent Diet20), ie, 100 mM, the same as two sugar cubes/cup. Sucrose has saturation point at 200gr/100mL (5.8M) [43]. Sucrose concentrations used in our study are labeled grey. **(b)** In ad lib mice, sucrose is preferred to sucralose (62±6% preference, n=8) at the maximal concentrations of both dynamic ranges, respectively, 140 mM and 1.5 mM. For concentrations outside of the dynamic ranges, 300 mM and 600 mM is preferred to 1 mM sucralose (98.8±0.5% and 98.7±0.8% preference, n=3), and 2.8M sucrose is preferred to 10 mM sucralose (85±4% preference, n=6). If comparing an infra-plateau concentration of sucrose (140 mM) with a plateau concentration of sucralose (10 mM) sucrose is iso-preferred (48±11%, n=6), but possibly due to saturation of T1R2/3 receptor, as EC_{50} for sucralose is much lower than that of sucrose [34,44-46], what is likely to prevent sucrose's access to the receptor. Procedural details in Supplemental Figure 5 and methods section.

Supplemental Fig.5. Tables containing lick data referring to SupplementalFig. 4b. For each concentration, the left column of numbers displays licks on the sucrose side, and the right column of numbers displays licks on the sucralose side. In all cases, n = m x r – i, where m= number of mice, r=number of run/mice, i= Zero/zero runs, from which a preference ratio (0/0+0) cannot be calculated. For each concentration X mM of sucrose, and Y mM of sucralose, (X,Y,m,r,i) = (300,1,3,1,0), (600,1,3,1,0), (140,1.5,2,4,0), (140,10, 2,4,0), (2.8,10,2,4,2). The mice in the group comparing 300 mM or 600 mM sucrose vs 1 mM sucralose are different from those in the remaining groups.

Supplemental Fig.6. ChR2+ mice in Figure3 lick at least 4.6 times more

sucrose than their daily intake from chow, and 10-min intake of sucrose, but not sucralose, is sufficient to raise blood glucose, even with low lick count. (a) MedAssociates lickometers lick volume = 2±0.2uL [47]. If corrected for body weight ratio, 1 lick translates into 1.6 teaspoons in humans. A co mMon emergency treatment for hypoglycemic patients is 3 teaspoons of sucrose in one cup (143 mM), waiting up to 15' for complete recovery*:* *(*http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001423). In mice, this would be equivalent to 60 licks of a 143 mM sucrose, if adjusting for mouse/human body weight ratio, and lick volume. **(b)** On average, sucrose intake of Chr2+ mice on Fig.3 was at least 4.2 times higher than an average daily chow intake . The table used the values in Supplemental Table1, referring to Chr2+ mice on the left panel of Fig.3. **(c)** Mice licked 140 mM sucrose for 10 min, and their blood glucose (BG) was measured 30minutes before the trial onset (*pre*), and at trial off- set (*post*). The same was done 2 days after for 0.5 mM sucralose in the same mice (2 mice were excluded due to shorter tail lengh). The data was sorted according to lick performance, and the BG post/pre ratios were averaged. On average, mice that licked sucrose less than 51 times increased BG to 132±10% (n=29) of the *pre* trial BG value, whereas if licking sucralose, BG was maintained at 101±4% (n=8). Mice that licked sucrose more than 50 times increased BG to 159.4±15% (n=12) of the *pre* trial BG value, whereas if licking sucralose, the BG was maintained at 109±2% (n=23).

Supplemental Fig.7. ChR2+ mice in Figure3 lick as much as those in Figure2, and tend to lick less later in the active phase (dark cycle-DC), but with invariant preference choice. (a) total number of licks (2 bottle were added) for all of the animals in each experimental group in Fig.2-3, normalized for 10 minutes. Only sucralose+laser vs water condition performed below the average of the other 3 experimental conditions, but had only 25% of data points collected in the 1st halves of the DC. **(b)** Circadian analysis of water+laser vs water data set in Fig.3 (Supplementary Fig. 7), which had 51/49% data points collected in the 1st/2nd halves of the DC. Regression analysis shows as significant

negative correlation between DC time and number of licks. In DC's 1st half mice licked a mean of 233±51.8 licks (n=11), and 90±38 licks in the 2nd half of DC (n=10). (**p<0.02). Preference ratios towards the non-laser side in the 1st/2nd halves of the Dc were, respectively, 28±5.8% and 32±5.8%. **(c)** New animals (m=4) were implanted and run as in fig.3-right panel exclusively in the 1st half of the DC, when mice licked more (see raster in bottom panel). The effect is comparable to that of Fig.3: the newly implanted animals (right panel) ,at most, isoprefer sucralose to water+laser (lick tables supp.Table5). Preference averages for 0 mM -1.5 mM sucralose are: 10±2.4% (n=4, *p<0.0001), 27.7±11% (n=6, *p<0.03), 41±15% (n=6, *p<0.04), 48.2±15% (n=6, *p<0.05), 48.5±13%(n=6, *p<0.03), 45±13%.(n=6,*p>0.09).

Supplemental Fig.8. Tables containing lick data referring to Fig. 3. For each concentration, the left column of numbers displays licks on the laser side, and the right column of numbers displays licks on the sweetener side., In all cases, n = m x r – i, where m= number of mice, r=number of run/mice, i= Zero/zero runs, from which a preference ratio (0/0+0) cannot be calculated. For Chr2- and for each concentration X of sucrose, (X,m,r,i)= (0, 4,6,1), (100, 4,3,2), (110, 4,3,3), (120, 4,3,2), (130, 4,4,3), (140, 4,3,2): Chr2+: (0, 5,5,4), (100, 5,2,0), (110,5,2,0), (120, 5,2,0), (130, 5,3,2), (140, 5,2,2). For Chr2+ and for each concentration Y of sucralose, (Y,m,r,i)= (0.125, 4,4,3), (0.25, 4,3,1), (0.5, 4,3,1), (1, 4,3,0), (1.5, 4,3,0): Chr2+: (0.125, 5,3,1), (0.25, 5,3,1), (0.5, 5,3,4), (1, 5,3,5), (1.5, 5,2,2)

Supplemental Fig.9. Tables containing lick data referring to Fig. 4. For each concentration, the left column of numbers displays licks on the 0.5 mM sucralose+laser side, and the right column of numbers displays licks on the sucrose side. In all cases, n = m x r – i, where m= number of mice, r=number of run/mice, i= Zero/zero runs, from which a preference ratio (0/0+0) cannot be calculated. For Chr2- and for each concentration X of sucrose, (X,m,r,i)= (110, 4,2,2), (140, 4,3,3): Chr2+: (100, 5,2,1), (140, 5,2,1).

Supplemental Fig.10. Tables containing lick data referring to Fig. 5. For each concentration, the left column of numbers displays licks on the 0.5 mM sucralose+laser side, and the right column of numbers displays licks on the sucrose side. In all cases, n = m x r – i, where m= number of mice, r=number of run/mice, i= Zero/zero runs, from which a preference ratio (0/0+0) cannot be calculated. For each concentration X of sucrose , "fast+IP veh"(X,m,r,i)= (110, 6,1,0), (140, 6,1,0) and , "fast+IP Lep"(X,m,r,i)= (110, 6,1,0), (140, 6,1,0)

Supplemental Fig.11. Optogenetic activation of DA neurons is invariant across metabolic states, and preferred flavor has higher DA activation. Left panel: Colocalization of nu-c-Fos/YFP on Dat-cre;Rosa26YFP/AAVDIOChR2mCherry shows that lick-induced optogenetic activation of DA neurons is invariant across metabolic states (adlib=14.8±1.9, fast=17.2±2.3, lep+=15±2.3, n=5). **Right panel:** In fasted animals, sucrose resulted in a significantly more DA/cFos positive neurons vs. sucralose+laser. (dark blue bars, sucrose and sucralose+laser activated, respectively, 49.4±4 and 34.8±4 DA neurons per 5122 pixel square, n=5, p(b)<0.0153). Conversely, upon leptin treatment, sucrose resulted in a significantly fewer DA/cFos vs. sucralose+laser. (green bars, sucrose and sucralose+laser activated, respectively, 11.8±2 and 32±2 DA neurons per 5122 pixel square, n=5, p(c)<0.00011). Light blue bars (ab libitum), are the same as in Fig.4, right panel.

Supplemental Fig.12. Tables containing lick data referring to Fig. 6. For each concentration, the left column of numbers displays licks on the 0.5 mM sucralose+laser side, and the right column of numbers displays licks on the sucrose side. In all cases, n = m x r – i, where m= number of mice, r=number of run/mice, i= Zero/zero runs, from which a preference ratio (0/0+0) cannot be calculated. For each concentration X of sucrose , "fast+ICV veh"(X,m,r,i)= (110, 6,1,0), (140, 6,1,0) and , "fast+ICV Lep"(X,m,r,i)= (110, 6,1,0), (140, 6,1,0).

Supplemental Fig.13. Increased blood glucose is not due to the gavage

procedure. Fasted Dat-Cre;Rosa26YFP animals were gavaged with 0.5ml of water (orange, yellow bars) (blue, green bars, same as in Fig.7). Blood glucose of leptin and vehicle treated animals gavaged with water were, respectively, 94±5 and 102±6 mg/dL; the difference is not statistically significant ($p>0.15$)

Supplemental Fig.14. Leptin regulates the value of sucrose, and regulates it's rewarding post-ingestive effect. Schematic model summarizing our findings. Animals make a choice between two sippers allowing to quantify the value of nutrients relative to lick-induced optogenetic stimulation of DA neurons. We show that leptin regulates the value of sucrose, and regulates it's post-ingestive effect.