

Supplemental Figure: Protocol for Infant Behavior Assessment Scale (IBAS), including the behavior tested, how it was measured, the optimal response (i.e., responses for which the subject would receive a score of 1, see text for details), other responses (i.e., responses for which the subject would receive a score of 0) and category of response.

Testing order	Behavior	How measured	Optimal response	Other responses	Category
1	Grasp	Observer rubs finger on infant's hands and feet	Strong grasp	None, weak	Basic Motor reflex
2	Sucking reflex	Nipple inserted into infant's mouth	Strong sucking response	None, weak	Survival
3	Startle response	Metal lid is dropped from 3 cm to counter top, behind infant's head	Startle response (e.g., eye blink) present	No startle response	Survival
4	Auditory orientation	Observer lip-smacks approximately 3 inches from infant's ear (both ears)	Orients quickly (2-3sec)	Orients slowly	Sensory
5	Orient to toy (near)	Toy held approximately 12 inches from infant's face for 10 sec	Direct eye contact to toy	Partial or no eye contact	Sensory
	Follow toy (near)	Toy is moved to right and left after 10 sec	Complete follow	Partial or no follow	Sensory
	Attention to toy (near)	Observer notes how much attention time infant attended to toy	At least 75% of time	Less than 75% of time	Sensory
6	Orient to toy (far)	Toy held approximately 24 inches from infant's face for 10 sec	Direct eye contact to toy	Partial or no eye contact	Sensory
	Follow toy (far)	Toy is moved to right and left after 10 sec	Complete follow	Partial or no follow	Sensory
	Attention to toy (far)	Observer notes how much attention time infant attended to toy	At least 75% of time	Less than 75% of time	Sensory
7	Feet placement	Infant is held with feet 1cm above countertop, and moved towards counter	Infant attempts to put feet on counter	Infant slow to put feet on counter	Basic Motor
8	Hand placement	Infant is held at 45 degree angle with head down, moved towards counter	Infant places hands on counter top	Infant slow to put hands on counter	Basic Motor
9	Clasping	Infant is placed on rolled up towel, which is then held approximately 6 inches off the counter. Towel is rotated so that infant is upside down.	Infant firmly holds towel (14 or 30 d), or climbs off (30 d).	Infant has a loose clasp or dangles from towel	Basic Motor
10	Righting	Infant is placed on its back on a towel. The infant is quickly released, and response to being placed in this position is noted.	Infant quickly rolls to stomach (i.e., rights)	Infant does not right self or rights self slowly (>5 sec)	Basic Motor

Supplemental Table: Statistics (mean, standard error, statistic (Kruskall Wallis H or ANOVA F) and p value) for behavioral tests. Asterisk signifies significant after Benjamini-Hochberg adjustment.

Test	Age	Behavior	Mean	SE	Statistic	p value	Notes
IBAS	14 days	Motor	ISO-3=2.38	0.50	H=1.68	0.43	
			ISO-1=2.75	0.75			
			CON=3.5	0.66			
		Survival	ISO-3=1.13	0.28	H=1.23	0.54	
			ISO-1=1.25	0.31			
			CON=0.88	0.23			
		Sensory	ISO-3=2.75	1.15	H=0.29	0.87	
			ISO-1=3.25	1.08			
			CON=2.50	0.73			
IBAS	30 days	Motor	ISO-3=2.63	0.53	H=7.89	0.02*	
			ISO-1=4.00	0.68			
			CON=5.25	0.49			
		Survival	ISO-3=1.50	0.19	H=0.78	0.68	
			ISO-1=1.25	0.31			
			CON=0.13	0.30			
		Sensory	ISO-3=2.75	0.77	H=1.91	0.39	
			ISO-1=1.50	0.57			
			CON=1.50	0.60			
Free play	3 months	Passive (first 5 min)	ISO-3=80.95	9.45	H=1.22	0.55	
			ISO-1=69.62	10.53			
			CON=8.54	10.96			
		Passive (last 5 min)	ISO-3=65.77	11.20	H=4.33	0.12	
			ISO-1=88.07	7.75			
			CON=65.51	12.80			
		Explore (first 5 min)	ISO-3=1.58	1.58	H=2.43	0.30	
			ISO-1=5.79	4.05			
			CON=4.91	3.45			
		Explore (last 5 min)	ISO-3=5.77	3.30	H=1.85	0.40	
			ISO-1=2.19	1.42			
			CON=8.19	3.38			
		Vocalization (first 5 min)	ISO-3=0.05	0.05	H=2.69	0.26	
			ISO-1=0.30	0.30			
			CON=0.93	0.56			
		Vocalization (last 5 min)	ISO-3=4.67	2.19	H=0.97	0.62	
			ISO-1=3.89	2.84			
			CON=6.64	3.19			
		Anxiety (last 5 min only)	ISO-3=0.65	0.33	H=2.19	0.33	
			ISO-1=0.86	0.86			
			CON=0.23	0.16			
		Leave dam					A
Human Intruder	3 months	Freeze (profile)	ISO-3=34.77	15.11	H=0.05	0.98	
			ISO-1=23.98	13.63			
			CON=32.27	13.78			
		Freeze (stare)	ISO-3=23.41	13.55	H=0.22	0.90	
			ISO-1=12.32	8.92			
			CON=18.24	8.32			

		Reaction (profile)	ISO-3=15.40 ISO-1=27.15 CON=23.50	10.44 11.60 12.21	H=0.97	0.62	
		Reaction (stare)	ISO-3=51.63 ISO-1=55.18 CON=57.09	15.74 11.76 13.47	$F_{(2,21)} = 0.04$	0.96	
		Anxiety					B
		Vigilance (profile)	ISO-3=61.91 ISO-1=57.40 CON=55.50	9.09 5.98 9.77	$F_{(2,21)} = 0.15$	0.86	
		Vigilance (stare)	ISO-3=81.65 ISO-1=63.93 CON=70.84	4.88 9.61 7.96	$F_{(2,21)} = 1.33$	0.29	
		Vocalize (profile)	ISO-3=4.07 ISO-1=6.20 CON=2.86	2.65 2.91 1.40	H=0.96	0.62	
		Vocalize (stare)	ISO-3=10.38 ISO-1=7.06 CON=6.86	4.23 1.85 2.27	H=0.15	0.93	
Human Intruder	12 months	Freeze (profile)	ISO-3=57.72 ISO-1=64.08 CON=61.56	10.75 7.36 10.82	$F_{(2,20)} = 0.11$	0.90	
		Freeze (stare)	ISO-3=24.11 ISO-1=20.32 CON=18.19	12.56 11.40 8.83	H=.01	0.99	
		Reaction (profile)					C
		Reaction (stare)	ISO-3=53.46 ISO-1=65.69 CON=47.92	19.43 15.91 13.49	$F_{(2,20)} = 0.33$	0.73	
		Anxiety					D
		Vigilance (profile)	ISO-3=22.99 ISO-1=46.60 CON=39.87	7.78 10.11 11.70	$F_{(2,20)} = 1.37$	0.28	
		Vigilance (stare)	ISO-3=68.81 ISO-1=66.43 CON=65.70	11.12 7.14 8.58	H=0.50	0.78	
Novel Object test	3 months	Latency (sec) to touch Kiwi	ISO-3=122.38 ISO-1=80.50 CON=95.25	42.91 43.06 40.39	H=0.44	0.80	
		Latency (sec) to touch toy	ISO-3=156.25 ISO-1=121.13 CON=166.75	41.79 51.45 51.47	H=0.48	0.79	
		Latency (sec) to touch Mr. Potato Head	ISO-3= 97.63 ISO-1=111.13 CON=124.75	37.24 46.19 41.23	H=0.47	0.79	
		Latency (sec) to touch snake	ISO-3= 58.13 ISO-1=38.75 CON=54.63	28.44 19.63 17.03	H=0.61	0.74	
		Take apple					E

Novel Object test	12 months	Latency (sec) to touch Kiwi	ISO-3=29.29	19.41	H=0.61	0.74	
			ISO-1=81.38	47.76			
			CON=13.88	8.23			
		Latency (sec) to touch toy	ISO-3=48.29	42.00	H=1.76	0.25	
			ISO-1=153.50	55.40			
			CON=37.63	12.57			
		Latency (sec) to touch Mr. Potato Head	ISO-3=132.71	59.17	H=0.60	0.74	
			ISO-1=155.00	50.89			
			CON=102.25	48.61			
		Latency (sec) to touch snake	ISO-3=86.14	40.23	H=5.96	0.05	
			ISO-1=192.75	42.83			
			CON=60.63	30.30			
		Latency (s) to take apple	ISO-3=49.29	41.85	H=0.52	0.77	
			ISO-1=64.50	37.56			
			CON=9.13	2.94			
		Hand in box					F
Home cage	12 months	Social behavior	ISO-3=20.45	9.41	$F_{(2,20)} = 0.22$	0.81	
			ISO-1=23.70	15.41			
			CON=20.40	7.36			
		Aggressive behavior	ISO-3=0.19	0.07	$F_{(2,20)} = 0.80$	0.47	
			ISO-1=0.18	0.05			
			CON=0.23	0.12			
		Anxiety behavior	ISO-3=0.38	0.16	H=8.41	0.02*	
			ISO-1=0.17	0.17			
			CON=0.23	0.07			
		Fear grimace	ISO-3=0.04	0.04	H=3.87	0.14	
			ISO-1=0.01	0.02			
			CON=0.02	0.02			
		Lip-smack	ISO-3=0.10	0.05	H=13.53	0.001*	
			ISO-1=0.01	0.02			
			CON=0.02	0.02			
		Yawn					G

Abbreviations:

ISO-1: Infants exposed to isoflurane one time

ISO-3: Infants exposed to isoflurane three times

CON: Controls (infants not exposed to isoflurane)

Notes:

A: Only 7 animals left their mother during the Freeplay test (2 ISO-3, 3 ISO-1, 2 controls; $X^2=0.40$, $df=2$, $p=0.82$)

B: Only two animals showed anxiety behavior during the Human Intruder test at 3 months.

C: No animal showed any behavioral response to the stranger during the profile period of the Human Intruder test at 3 months

D: No animal showed anxiety behavior during the Human Intruder test at 12 months

E: 12 animals took the apple from the snake (4 ISO-3, 5 ISO-1, 3 controls; $X^2=1.00$, $df=2$, $p=0.61$)

F: 7 animals put their hand in the black box (0 ISO-3, 3 ISO-1, 4 controls; $X^2=4.14$, $df=2$, $p=0.13$)

G: Only 6 animals yawned during the home cage behavioral observations (4 ISO-3, 1 ISO-1, 1 control; $X^2= 5.0$, $df=2$, $p=0.08$).