Limited replicability of drug-induced amnesia after contextual fear memory retrieval in rats

Natalie Schroyens, Joaquín Matias Alfei, Anna Elisabeth Schnell, Laura Luyten, Tom Beckers

-- Appendix A --
Supplementary Methods

Exp.	*N*	Strain	Enrich-ment	Acclim. (days)	Handling	Training						React. duration	Pre-react. Treatment	Post-react. Treatment
						Pre shock	Post shock	# shocks	Shock intensity	Shock duration	Training duration			
JA01[1]	15	SD	/	4	5 days (10 min)	3 min	30 s	2	.5 mA	2 s	4 min 4 s	4 min 4 s	/	PROP
JA02[1]	12	SD	/	2	4 days (12 min)	3 min	30 s	1	.5 mA	2 s	3 min 32 s	3 min 32/34 s	/	PROP
JA03	12	SD	/	1	5 days (15 min)	1 min	30 s	2	.4 mA	2 s	2 min 4 s	2 min 4 s	/	SAL, PROP, MDZ
JA04	12	SD	yes	2	4 days (16 min)	3 min	30 s	2	.4 mA	2 s	4 min 4 s	2 min	/	SAL, PROP, MDZ
JA05	12	SD	yes	2	4 days (16 min)	3 min	30 s	3	.4 mA	2 s	4 min 36 s	2 min	/	SAL, PROP, MDZ
JA06	12	SD	yes	6	4 days (16 min)	3 min	30 s	3	.4 mA	2 s	4 min 36 s	2 min	/	SAL, PROP, MDZ
JA07	11	SD	yes	5	4 days (16 min)	3 min	30 s	3	.4 mA	2 s	4 min 36 s	4 min 6 s	/	SAL, PROP, MDZ
JA08	16	SD	yes	2	4 days (16 min)	3 min	30 s	3	.4 mA	2 s	4 min 36 s	4 min 6 s	/	SAL, PROP, MDZ
NS01A	12	SD	/	6	/	3 min	30 s	2	.4 mA	2 s	4 min 4 s	4 min 4 s	/	SAL, PROP, MDZ
NS01B	12	SD	/	3	3 days (9 min)	3 min	30 s	2	.4 mA	2 s	4 min 4 s	4 min 4 s	/	SAL, PROP, MDZ
NS02	12	SD	yes	2	4 days (12 min)	1 min	30 s	2	.3 mA	2 s	2 min 4 s	2 min 4 s	/	SAL, PROP, MDZ
NS03	12	SD	yes	2	4 days (12 min)	3 min	30 s	2	.4 mA	2 s	4 min 4 s	3 min	/	SAL, PROP, no inj.
NS04	12	SD	yes	3	3 days (12 min)	3 min	30 s	3	.4 mA	2 s	4 min 36 s	2 min	/	SAL, PROP, MDZ
NS05	12	SD	yes	3	4 days (16 min)	3 min	30 s	3	.5 mA	2 s	4 min 36 s	2 min	/	SAL, PROP, MDZ
NS06	12	SD	yes	2	4 days (16 min)	3 min	30 s	3	.4 mA	2 s	4 min 36 s	5 min 36 s	/	SAL, MDZ
NS07	12	SD	yes	6	/	3 min	30 s	3	.4 mA	2 s	4 min 36 s	5 min 36 s	/	SAL, MDZ
NS08[2]	12	SD	yes	11	/	3 min	30 s	3	.4 mA	2 s	4 min 36 s	5 min 36 s	/	SAL, MDZ
NS10	12	W	yes	10	4 days (20 min)	1 min	0 s	2	1 mA	3 s	1 min 36 s	3 min	/	SAL, MDZ
NS12	14	W	yes	9	3 days (6 min)	1 min	0 s	2	1 mA	3 s	1 min 36 s	2 min	SAL, DCS	MDZ
NS13	16	W	yes	5	3 days (6 min)	1 min	0 s	2	1 mA	3 s	1 min 36 s	2 min	SAL	SAL, MDZ
NS14	16	W	yes	3	1 day (2 min)	1 min	0 s	2	1 mA	3 s	1 min 36 s	1 min	/	SAL, MDZ
NS17[2]	16	W	yes	7	4 days (4 min)	3 min	1 min	1	1 mA	3 s	4 min 3 s	4 min	/	SAL, MDZ
NS18	16	W	yes	15	3 days (3 min)	1 min	0 s	2	1 mA	3 s	1 min 36 s	2 min	SAL, MDZ	/
NS20[2]	32	W	yes	7	3 days (6 min)	3 min	1 min	1	1 mA	3 s	4 min 3 s	4 min	SAL, DCS	SAL, MDZ, CYLCO
NS21	16	W	yes	10	4 days (4 min)	1 min	0 s	2	1 mA	3 s	1 min 36 s	2 min	/	SAL, MDZ

Table A.1. Parameters of experiments investigating post-reactivation amnesia induction (conceptual replication attempts).

Several parameters have been manipulated throughout this series of 25 experiments, including training parameters (amount of shocks, shock intensity, amount of training sessions, pre- and post-shock period), duration of the reactivation session, amount of handling, rat strain, amnestic drug and dose, the use of cage enrichment, and the duration of the acclimatization period. Intertrial interval (ITI, time interval between shocks) was 30 s in all experiments.

[1] Prediction error (PE) during the reactivation session was manipulated. To this end, the reactivation session consisted of an exact repetition of the initial training session (no PE) or variations with respect to initial training by using a stronger shock (positive PE, JA01), more shocks (positive PE, JA02), or the omission of the shock (negative PE).

[2] Multiple training sessions were used in experiments NS08 (i.e., 2 sessions on 2 subsequent days), and NS17 and NS20 (i.e., 3 sessions on 3 subsequent days).

Explanation of column headers:

- **Exp.:** Experiment identifier. Each study is identified by using the initials of the responsible experimenter (NS or JA), followed by a number that indicates the chronological order in which the experiments were conducted. Experiments JA01-JA10 were performed by JA (who also performed the successful experiments in Alfei et al. (2015) and Ferrer Monti et al. (2017)). Experiments NS01-NS12 and NS14-NS17 were performed by NS. An undergraduate student assisted in conducting experiments NS06-NS12 and performed NS13. AS performed experiments NS18 and NS20.
- **Strain**: SD = male Sprague-Dawley rats (270 – 300 g at time of arrival in the lab), W = male Wistar rats (7-8 weeks old at time of arrival in the lab). Older Wistar rats (13-15 weeks at time of arrival in the lab) were used in experiment NS11 in order to directly replicate the successful study by Stern et al. (2012). Younger Wistar rats (6-7 weeks old at time of arrival in the lab) were used in experiment NS18, in order to allow for an extended acclimatization period (i.e., 18 days between arrival in the lab and conditioning).
- **Enrichment**: Cage enrichment, a tunnel hanging from the top grid, was provided in most of the experiments.
- **Acclim. (days):** Rats were left undisturbed in their home cages for 1-15 days prior to handling. Considering the amount of handling in each study, this implies that there was an interval of 6-18 days between arrival in the lab and the start of the fear conditioning protocol.
- **Handling**: Describes the number of days the animals were handled prior to conditioning and an approximation of the total handling time (i.e., all handling sessions combined).
- **Pre-react. Treatment**: In experiments NS12, NS13, and NS20, D-cycloserine (DCS, 15 mg/kg) or vehicle (saline, SAL) was administered 30 min before the reactivation session. In experiment NS18, midazolam (MDZ, 3 mg/kg) or vehicle (SAL) was administered 20 min before the reactivation session.
- **Post-react. Treatment**: Amnestic agents include propranolol (PROP, 10 mg/kg), midazolam (MDZ, 3 mg/kg or 10 mg/kg), and cycloheximide (CYCLO, 1.5 mg/kg). These drugs, or an equivalent amount of vehicle (SAL), were administered at a volume of 1 ml/kg immediately after the reactivation session. In experiment NS03, one group of rats received no injection ('no inj.').

Exp.	*N*	Strain	Enrich-ment	Acclim. (days)	Handling	Training						Reactivation duration	Post-react Treatment
						Pre shock	Post shock	# shocks	Shock intensity	Shock duration	Training duration		
JA09[1]	12	W	yes	3	4 days (20 min)	1 min	0 s	2	1 mA	3 s	1 min 36 s	2 min	SAL, MDZ
JA10[1]	15	W	/	3	4 days (20 min)	1 min	0 s	2	1 mA	3 s	1 min 36 s	2 min	SAL, MDZ
JA11[2]	20	W	/	11	4 days (20 min)	1 min	0 s	2	1 mA	3 s	1 min 36 s	2 min	SAL, MDZ
JA12[2]	19	W	/	14	4 days (20 min)	1 min	0 s	2	1 mA	3 s	1 min 36 s	2 min	SAL, MDZ
NS09[1]	12	W	yes	7	4 days (20 min)	1 min	0 s	2	1 mA	3 s	1 min 36 s	2 min	SAL, MDZ
NS11[3]	12	W	/	13	3 min CTX habituation	30 s	30 s	3	.7 mA	3 s	2 min 9 s	3 min	SAL, MDZ (1.5 mg/kg)

Table A.2. Attempts to exactly replicate previous reports of post-reactivation amnesia induction. Unless specified otherwise, the usual dose of 3 mg/kg MDZ was used in these experiments. See Table A.1 for explanation of column headers.

[1] Replications of Alfei et al. (2015) and Ferrer Monti et al. (2017) that were performed in two different lab spaces at KU Leuven (Belgium). JA10 was performed in a different lab space (compared to all other studies described here) to (partially) exclude the possibility that certain properties of our department's lab environment and housing facilities prevented us from obtaining amnesia.

[2] Replications of Alfei et al. (2015) and Ferrer Monti et al. (2017) that were performed in the same lab as the original studies (Universidad Nacional de Córdoba, Argentina).

[3] Replication of Stern et al. (2012), in which rats were not handled but habituated to the training context (CTX) for 3 min on the day prior to conditioning. A dose of 1.5 mg/kg MDZ was used.

Exp.	*N*	Strain	Enrich-ment	Acclim. (days)	Handling	Training						Reactivation duration	Treatment
						Pre shock	Post shock	# shocks	Shock intensity	Shock duration	Training duration		
N14	32	W	yes	3	1 day (2 min)	1 min	0 s	2	1 mA	3 s	1 min 36 s	1 min 30 min	SAL or MDZ (3 mg/kg) immediately after reactivation
N15	24	W	yes	1	5 days (5 min)	3 min	30 s	3	.4 mA	2 s	4 min 36 s	/	SAL or MDZ (1.5 or 3 mg/kg) 20 min before learning

Table A.3. Midazolam (MDZ) administration after extinction (NS14) or before conditioning (NS15): Effects on locomotor activity and fear memory retention. See Table A.1 for explanation of column headers.

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-- Appendix B --
Descriptive Statistics and Results of Statistical Analyses

The following tables present the descriptive statistics and results of statistical analyses for all experiments in which midazolam (MDZ) or propranolol (PROP) was administered systemically after re-exposure to the conditioning context (versus saline, SAL). One-sided (frequentist and Bayesian) t-tests were performed to assess whether MDZ- or PROP-treated rats showed less freezing during the retention test, compared to SAL rats. Based on the labels proposed by Jeffreys (1961), a BF_{10} between .33 and 1 suggests anecdotal evidence in favor of H_0 (i.e., the absence of an amnestic effect), while a BF_{10} smaller than .33 suggests substantial evidence in favor of H_0. A BF_{10} between 1 and 3 suggests anecdotal evidence in favor of H_A (i.e., the presence of an amnestic effect).

	Descriptive Stats						Results Statistical Analyses					
	SAL			MDZ						95% CI for d		
Exp.	***n***	**mean**	**SD**	***n***	**mean**	**SD**	**t**	**p**	**Cohen's d**	**lower**	**upper**	**BF_{10}**
JA03	4	67.33	21.22	2	55.00	16.97	-0.70	0.260	-0.61	-2.32	1.17	0.90
JA04	4	30.33	23.69	3	37.44	6.05	0.50	0.680	0.38	-1.15	1.88	0.42
JA05	3	61.56	18.17	4	56.25	14.83	-0.43	0.343	-0.33	-1.82	1.20	0.71
JA06	3	30.11	22.70	3	43.31	32.66	0.57	0.702	0.47	-1.19	2.07	0.43
JA07	4	51.33	11.97	4	37.33	19.45	-1.23	0.133	-0.87	-2.30	0.63	1.27
JA08	5	41.00	8.64	5	60.60	18.22	2.17	0.969	1.37	-0.06	2.75	0.23
NS01A	4	44.42	29.93	4	64.92	18.75	1.16	0.855	0.82	-0.67	2.25	0.32
NS01B	4	36.17	15.15	4	34.58	9.34	-0.18	0.432	-0.13	-1.51	1.27	0.58
NS02	3	6.44	4.60	2	21.33	1.41	4.24	0.988	3.87	0.40	7.27	0.31
NS04	4	49.67	28.90	4	55.83	24.36	0.33	0.622	0.23	-1.17	1.61	0.44
NS05	4	28.25	17.76	4	57.17	16.65	2.38	0.972	1.68	-0.03	3.30	0.25
NS06	5	43.27	18.37	6	39.11	16.64	-0.39	0.351	-0.24	-1.42	0.96	0.62
NS07	4	39.75	16.93	5	27.40	20.63	-0.96	0.184	-0.65	-1.98	0.73	1.01
NS08	6	48.11	31.56	6	53.67	21.53	0.36	0.635	0.21	-0.93	1.34	0.38
NS10	6	60.11	9.78	6	67.06	21.60	0.72	0.755	0.41	-0.74	1.55	0.32
NS13	8	72.00	14.97	8	56.83	17.14	-1.88	**0.040**	-0.94	-1.97	0.11	2.42
NS14	8	79.71	16.51	8	66.17	20.48	-1.46	0.084	-0.73	-1.73	0.30	1.48
NS17	8	76.54	13.58	8	78.67	13.27	0.32	0.622	0.16	-0.83	1.14	0.35
NS20	8	70.67	19.17	8	69.17	21.62	-0.15	0.443	-0.07	-1.05	0.91	0.47
NS21	8	58.00	27.38	8	79.50	10.41	2.08	0.972	1.04	-0.03	2.07	0.18

Table B.1. Descriptive statistics (sample size, mean percentage freezing, SD) for each group and results of statistical analyses of 20 conceptual replication attempts in which midazolam (MDZ) or saline (SAL) was administered after re-exposure to the conditioning context. Based on a pre-defined criterion, rats were excluded if they showed less than 25% freezing during the reactivation session. The Bayes factor (BF_{10}) quantifies evidence in favor of the alternative hypothesis (H_A; i.e., MDZ < SAL) relative to the null hypothesis (H_0). A BF_{10} between .33 and 1 suggests *anecdotal evidence in favor of H_0 (i.e., the absence of an amnestic effect)*, while a BF_{10} smaller than .33 suggests *substantial evidence in favor of H_0*. A BF_{10} between 1 and 3 suggests *anecdotal evidence in favor of H_A (i.e., the presence of an amnestic effect)*.

	Descriptive Stats						Results Statistical Analyses					
	SAL			PROP						95% CI for d		
Exp.	***n***	**mean**	**SD**	***n***	**mean**	**SD**	**t**	**p**	**Cohen's d**	**lower**	**upper**	**BF_{10}**
JA03	4	67.33	21.22	4	46.67	32.49	-1.07	0.164	-0.75	-2.17	0.72	1.11
JA04	4	30.33	23.69	4	41.42	22.35	0.68	0.739	0.48	-0.95	1.87	0.37
JA05	3	61.56	18.17	4	53.58	16.35	-0.61	0.284	-0.47	-1.97	1.08	0.81
JA06	3	30.11	22.70	2	40.20	6.41	0.58	0.700	0.53	-1.34	2.33	0.45
JA07	4	51.33	11.97	3	40.00	15.41	-1.10	0.160	-0.84	-2.39	0.77	1.17
JA08	5	41.00	8.64	6	43.28	29.06	0.17	0.565	0.10	-1.09	1.29	0.44
NS01A	4	44.42	29.93	4	43.25	6.79	-0.08	0.471	-0.05	-1.44	1.33	0.55
NS01B	4	36.17	15.15	3	25.89	9.26	-1.03	0.176	-0.78	-2.32	0.82	1.10
NS02	3	6.44	4.60	3	40.56	31.34	1.87	0.932	1.52	-0.44	3.36	0.31
NS03	4	45.33	35.08	4	21.25	5.85	-1.35	0.132	-0.96	-2.41	0.56	1.41
NS04	4	49.67	28.90	4	66.58	17.28	1.00	0.823	0.71	-0.76	2.12	0.33
NS05	4	28.25	17.76	4	60.33	16.10	2.68	0.982	1.89	0.11	3.58	0.24

Table B.2. Descriptive statistics (sample size, mean percentage freezing, SD) for each group and results of statistical analyses of 12 conceptual replication attempts in which propranolol (PROP) or saline (SAL) was administered after re-exposure to the conditioning context. Based on a pre-defined criterion, rats were excluded if they showed less than 25% freezing during the reactivation session. The Bayes factor (BF_{10}) quantifies evidence in favor of the alternative hypothesis (H_A; i.e., PROP < SAL) relative to the null hypothesis (H_0). A BF_{10} between .33 and 1 suggests *anecdotal evidence in favor of H_0 (i.e., the absence of an amnestic effect)*, while a BF_{10} smaller than .33 suggests *substantial evidence in favor of H_0*. A BF_{10} between 1 and 3 suggests *anecdotal evidence in favor of H_A (i.e., the presence of an amnestic effect)*.

	Descriptive Stats						Results Statistical Analyses					
	SAL			MDZ						95% CI for d		
Exp.	***n***	**mean**	**SD**	***n***	**mean**	**SD**	**t**	**p**	**Cohen's d**	**lower**	**upper**	**BF_{10}**
JA09	6	66.22	11.48	6	59.00	9.32	-1.20	0.130	-0.69	-1.84	0.50	1.18
JA10	7	62.86	13.68	8	58.96	11.03	-0.61	0.276	-0.32	-1.33	0.71	0.68
JA11	10	63.00	18.07	10	47.17	22.05	-1.76	**0.048**	-0.79	-1.69	0.14	2.07
JA12	9	58.96	22.44	10	40.90	18.86	-1.91	**0.037**	-0.88	-1.81	0.08	2.50
NS09	6	61.33	30.69	6	70.67	15.04	0.67	0.741	0.39	-0.77	1.52	0.33
NS11	6	63.67	26.19	6	70.50	13.31	0.57	0.709	0.33	-0.82	1.46	0.34

Table B.3. Descriptive statistics (sample size, mean percentage freezing, SD) for each group and results of statistical analyses of 6 exact replication attempts in which midazolam (MDZ) or saline (SAL) was administered after re-exposure to the conditioning context. The Bayes factor (BF_{10}) quantifies evidence in favor of the alternative hypothesis (H_A; i.e., MDZ < SAL) relative to the null hypothesis (H_0). A BF_{10} between .33 and 1 suggests *anecdotal evidence in favor of H_0 (i.e., the absence of an amnestic effect)*, while a BF_{10} smaller than .33 suggests *substantial evidence in favor of H_0*. A BF_{10} between 1 and 3 suggests *anecdotal evidence in favor of H_A (i.e., the presence of an amnestic effect)*.

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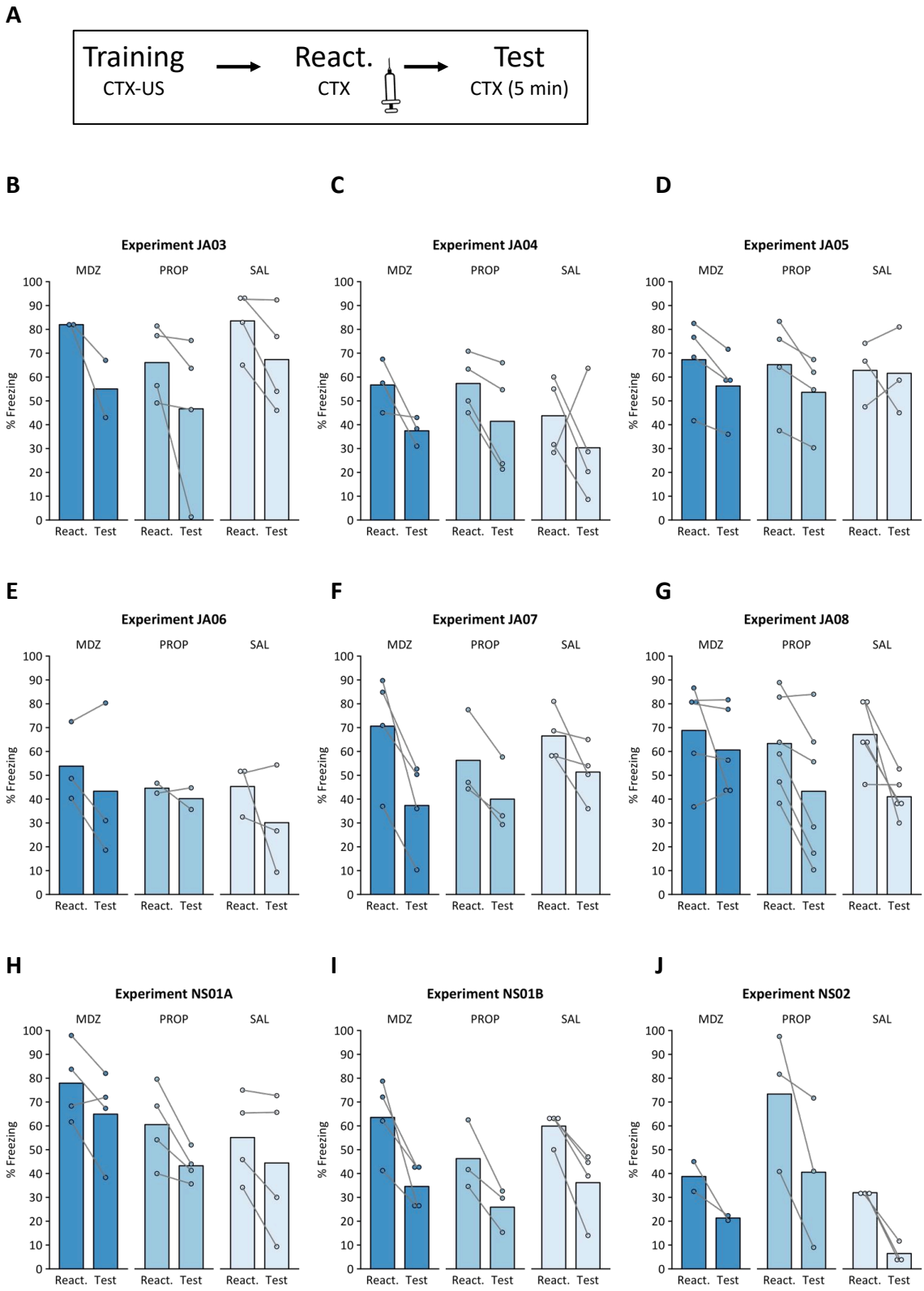
-- Appendix C --
Freezing during the Reactivation Session and Retention Test


A
Training
CTX-US
React.
CTX
Test
CTX (5 min)
B
Experiment JA03
C
Experiment JA04
D
Experiment JA05
E
Experiment JA06
F
Experiment JA07
G
Experiment JA08
H
Experiment NS01A
I
Experiment NS01B
J
Experiment NS02
MDZ
PROP
SAL
% Freezing
React.
Test

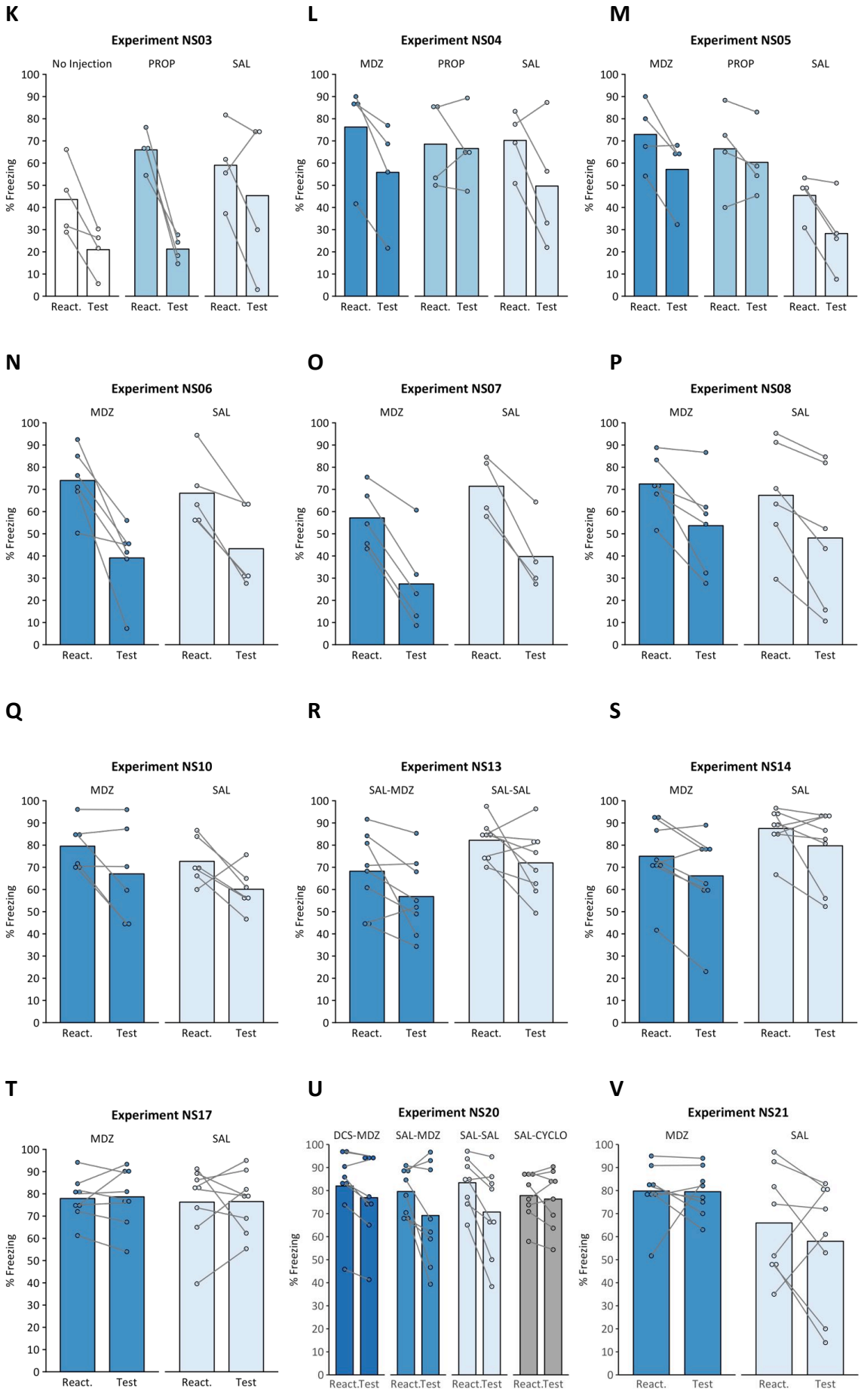

K
Experiment NS03
No Injection
PROP
SAL
L
Experiment NS04
MDZ
PROP
SAL
M
Experiment NS05
MDZ
PROP
SAL
N
Experiment NS06
MDZ
SAL
O
Experiment NS07
MDZ
SAL
P
Experiment NS08
MDZ
SAL
Q
Experiment NS10
MDZ
SAL
R
Experiment NS13
SAL-MDZ
SAL-SAL
S
Experiment NS14
MDZ
SAL
T
Experiment NS17
MDZ
SAL
U
Experiment NS20
DCS-MDZ
SAL-MDZ
SAL-SAL
SAL-CYCLO
V
Experiment NS21
MDZ
SAL
% Freezing
React.
Test
React.Test

Figure C.1. Twenty-one failures to conceptually replicate post-reactivation amnesia induction for contextual fear memories. Individual data and group means are shown. Rats showing less than 25% freezing during the reactivation session were excluded. SAL = saline; MDZ = midazolam; PROP = propranolol; CYCLO = cycloheximide; DCS = D-cycloserine. **(A-Q, S-T, V)** Drugs were administered systemically, immediately after the reactivation session (React.) and fear memory retention was assessed 24 h later (Test, 5 min). **(R)** SAL was administered 30 min before the reactivation session, and SAL or MDZ immediately after the reactivation session. This experiment was carried out as a follow-up to experiment NS12 (see Fig. C.4B). **(U)** DCS or SAL was administered 30 min before the reactivation session, and SAL, MDZ, or CYCLO immediately after the reactivation session. An overview of training and reactivation parameters for each study can be found in Appendix A (Table A.1).

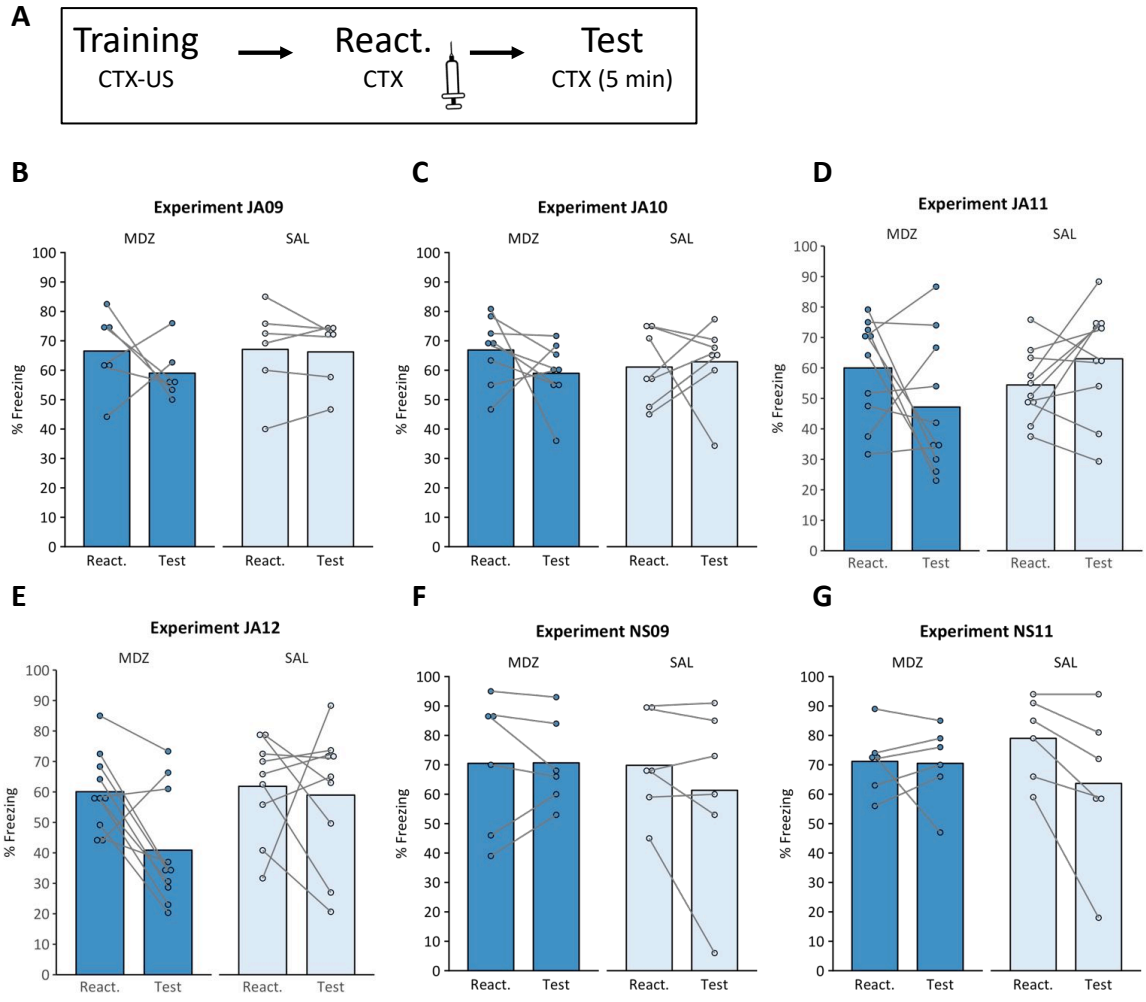


Figure C.2. Six attempts to exactly replicate post-reactivation amnesia induction for contextual fear memories. Individual data and group means are shown. **(A)** Midazolam (MDZ) or saline (SAL) was administered systemically, immediately after the reactivation session ('React.') and fear memory retention was assessed 24 h later (Test, 5 min). Exact replication attempts following the methodology of **(B-F)** Alfei et al. (2015) and Ferrer Monti et al. (2017) or **(G)** Stern and colleagues (2012). An overview of training and reactivation parameters for each study can be found in Appendix A (Table A.2).

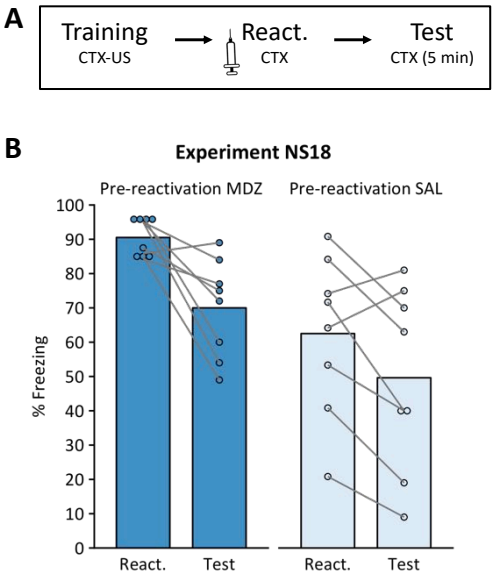


Figure C.3. No influence of pre-reactivation MDZ administration on fear memory retention. Individual data and group means are shown. **(A)** Midazolam (MDZ) or saline (SAL) was administered 20 min before the reactivation session (React.) and fear memory retention was assessed 24 h later (Test, 5 min). **(B)** MDZ acutely enhanced freezing during the reactivation session due to an MDZ-induced decrease in locomotor activity. As a result, the change in % freezing from the reactivation session (under influence of MDZ) to test (drug-free state) in MDZ rats cannot be interpreted unambiguously. Although visual inspection of the graphs suggests that MDZ rats showed higher fear memory retention compared to SAL rats at test, this difference did not reach statistical significance.

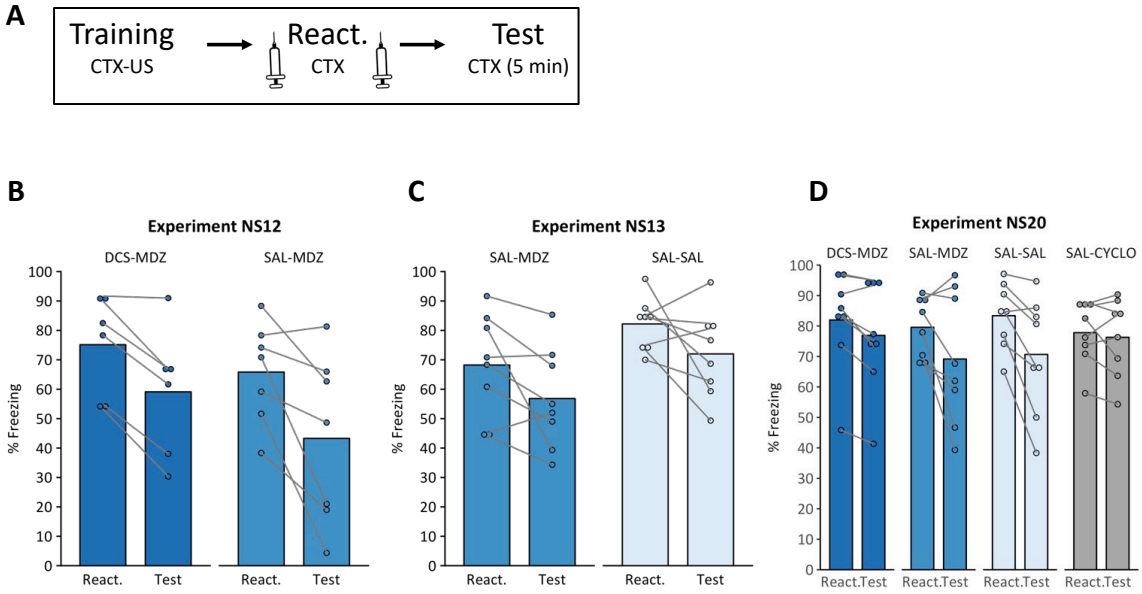


Figure C.4. No influence of pre-reactivation DCS administration on fear memory malleability. Individual data and group means are shown. **(A)** D-cycloserine (DCS) or saline (SAL) was administered 30 min before the reactivation session (React.), and midazolam (MDZ), cycloheximide (CYCLO) or SAL after React. Fear memory retention was assessed 24 h later (Test, 5 min). **(B-D)** Post-reactivation MDZ or CYCLO administration did not induce amnesia, whether or not DCS was administered before the reactivation session in an attempt to boost memory destabilization.

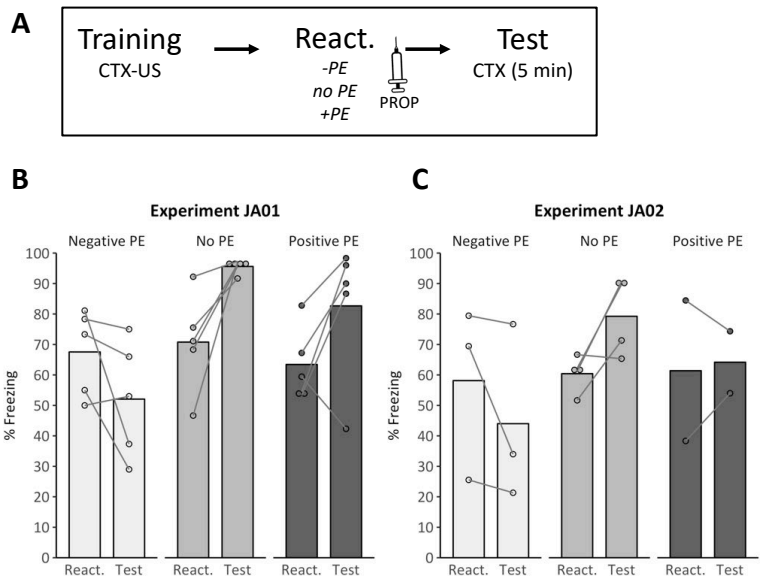


Figure C.5. Manipulating prediction error during the reactivation session did not allow for the induction of post-reactivation amnesia. Individual data and group means are shown. **(A)** Propranolol (PROP) was administered systemically after the reactivation session (React.) and memory retention was assessed 24 h later (Test). **(B)** During React., animals were either retrained using the same parameters as during training (no prediction error, 'no PE') or using a higher shock intensity ('Positive PE'), or the shock was omitted ('Negative PE'). **(C)** During React., animals were either retrained using the same parameters as during training ('no PE') or using more shocks (i.e., 2 shocks instead of 1 shock, 'positive PE'). In the third group, the shock was omitted ('negative PE').

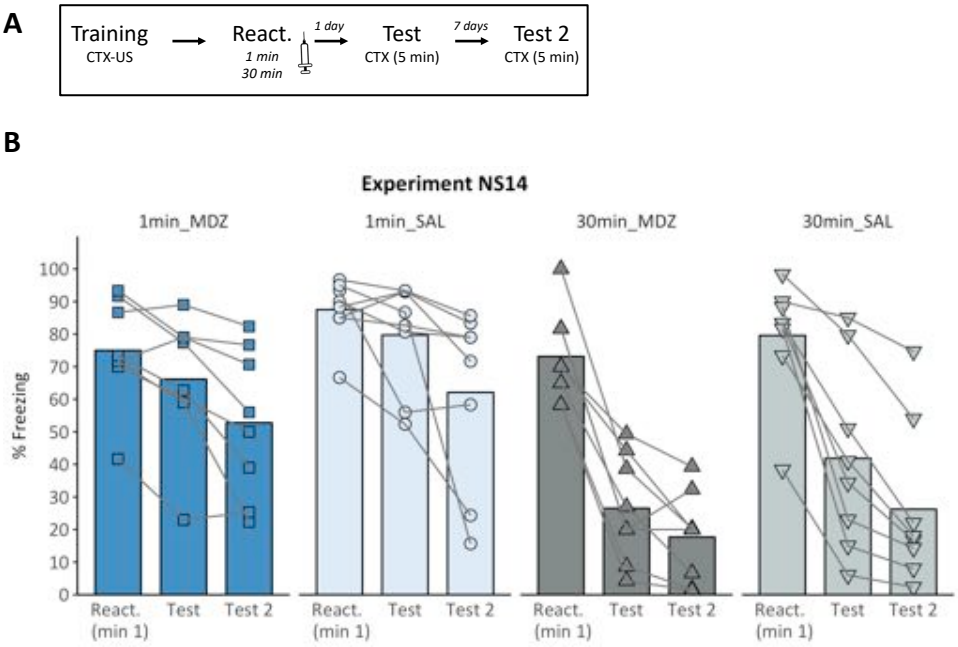


Figure C.6. MDZ administration after short or long memory reactivation session did not affect fear memory retention. Individual data and group means are shown. **(A)** Midazolam (MDZ) or saline (SAL) was administered

systemically after a brief (1 min) or long (30 min) memory reactivation session. Fear memory retention was assessed 1 (Test, 5 min) and 8 days later (Test 2, 5 min). **(B)** Post-reactivation MDZ administration did not affect fear memory retention.

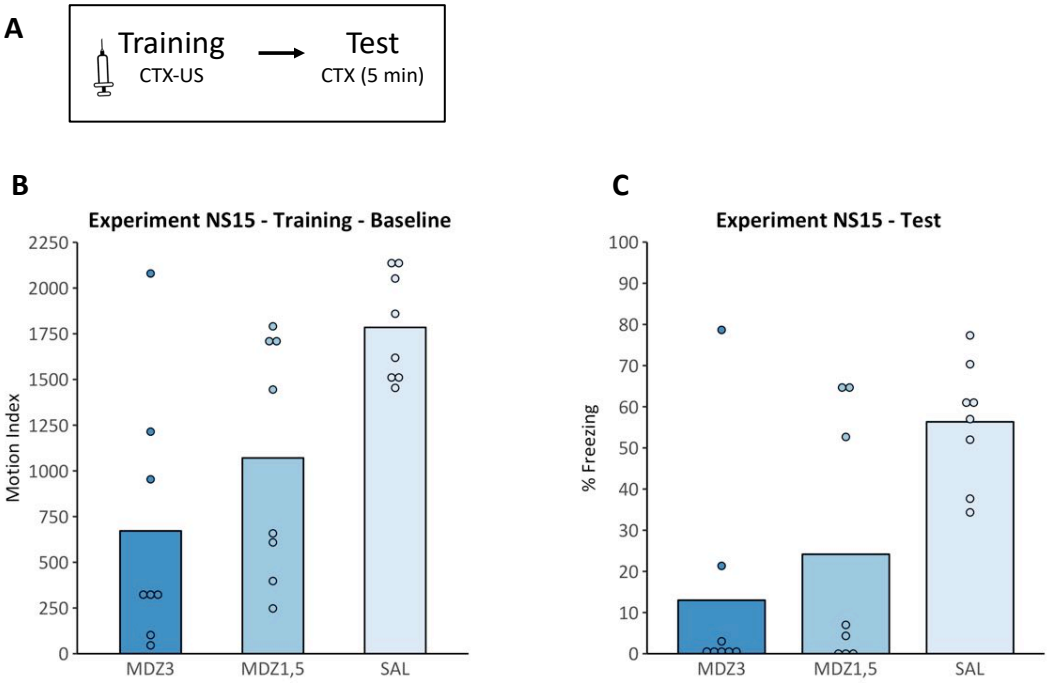
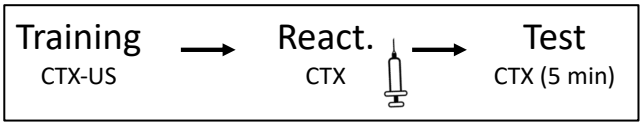


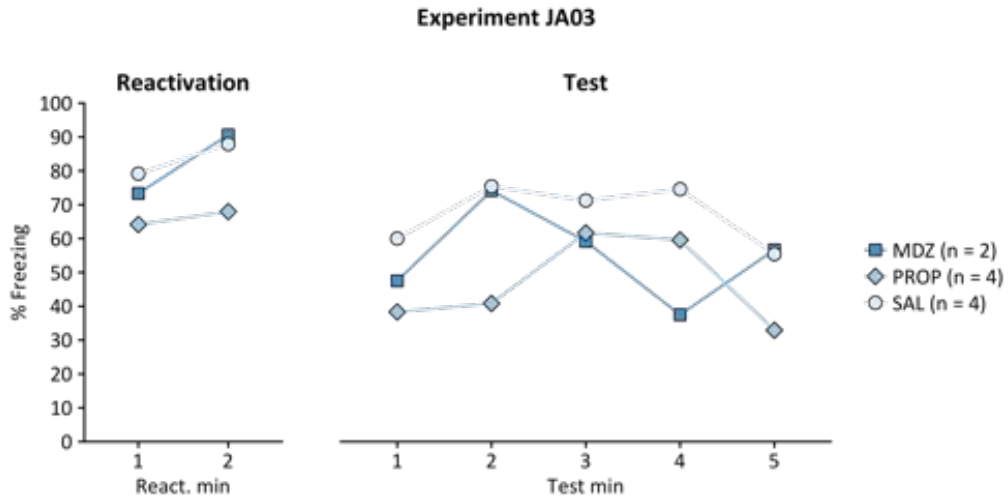
Figure C.7. MDZ administration before contextual fear conditioning acutely impairs locomotor activity and results in lower fear memory expression one day later. Individual data and group means are shown. **(A)** Midazolam (MDZ) or saline (SAL) was administered systemically 20 min before contextual fear conditioning. **(B)** MDZ induced a dose-dependent impairment in locomotor activity during the 3-min baseline period of the training session, as evidenced by a decrease in motion index (MedAssociates software). **(C)** MDZ administration before learning impaired subsequent fear memory retention, as evidenced by lower freezing scores during a context exposure session one day later.

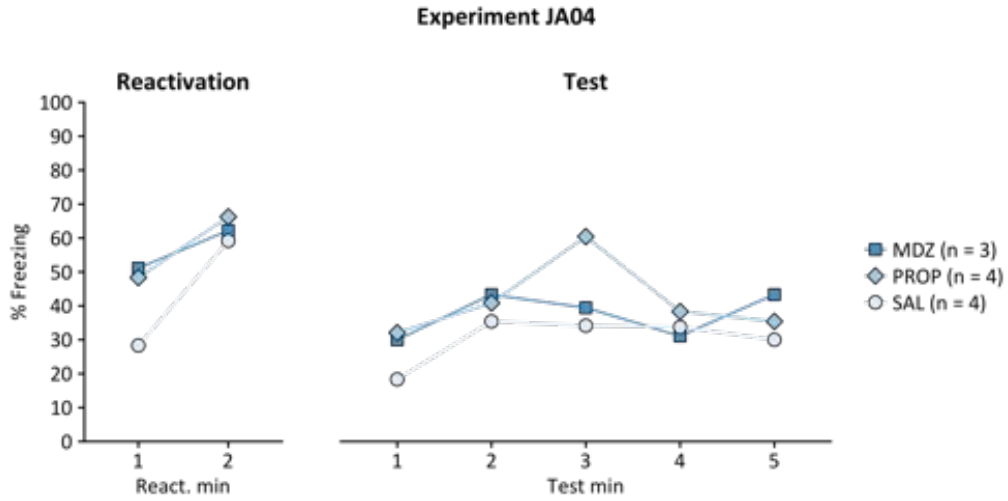
Limited replicability of drug-induced amnesia after contextual fear memory retrieval in rats

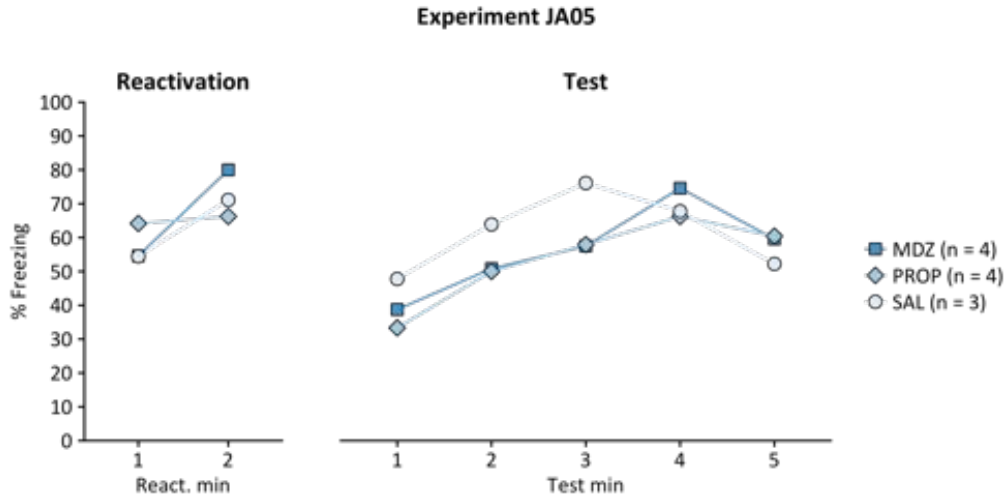
Natalie Schroyens, Joaquín Matias Alfei, Anna Elisabeth Schnell, Laura Luyten, Tom Beckers

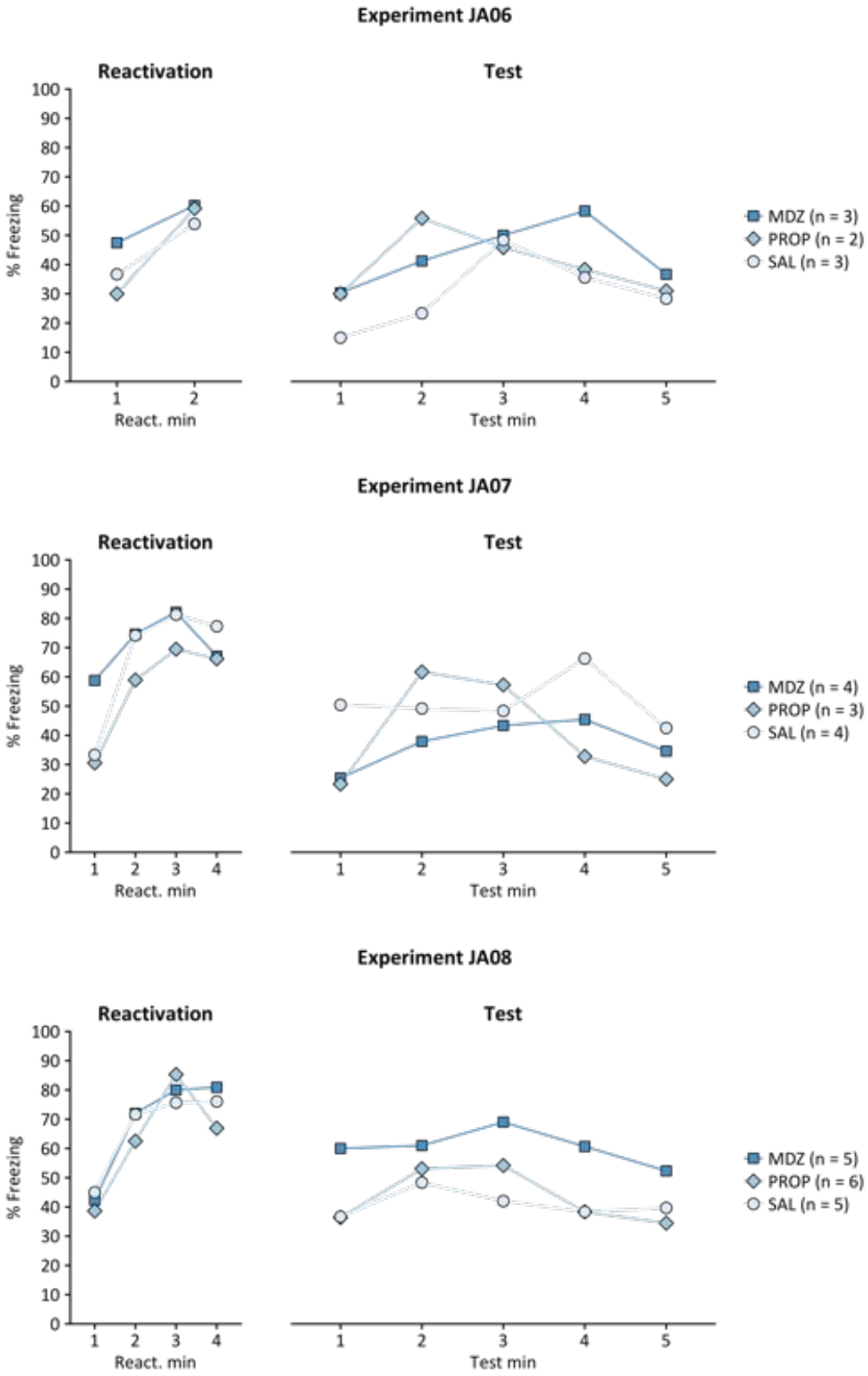
-- Appendix D --
Temporal Patterns of Contextual Fear:
Freezing per Minute during Reactivation and Test

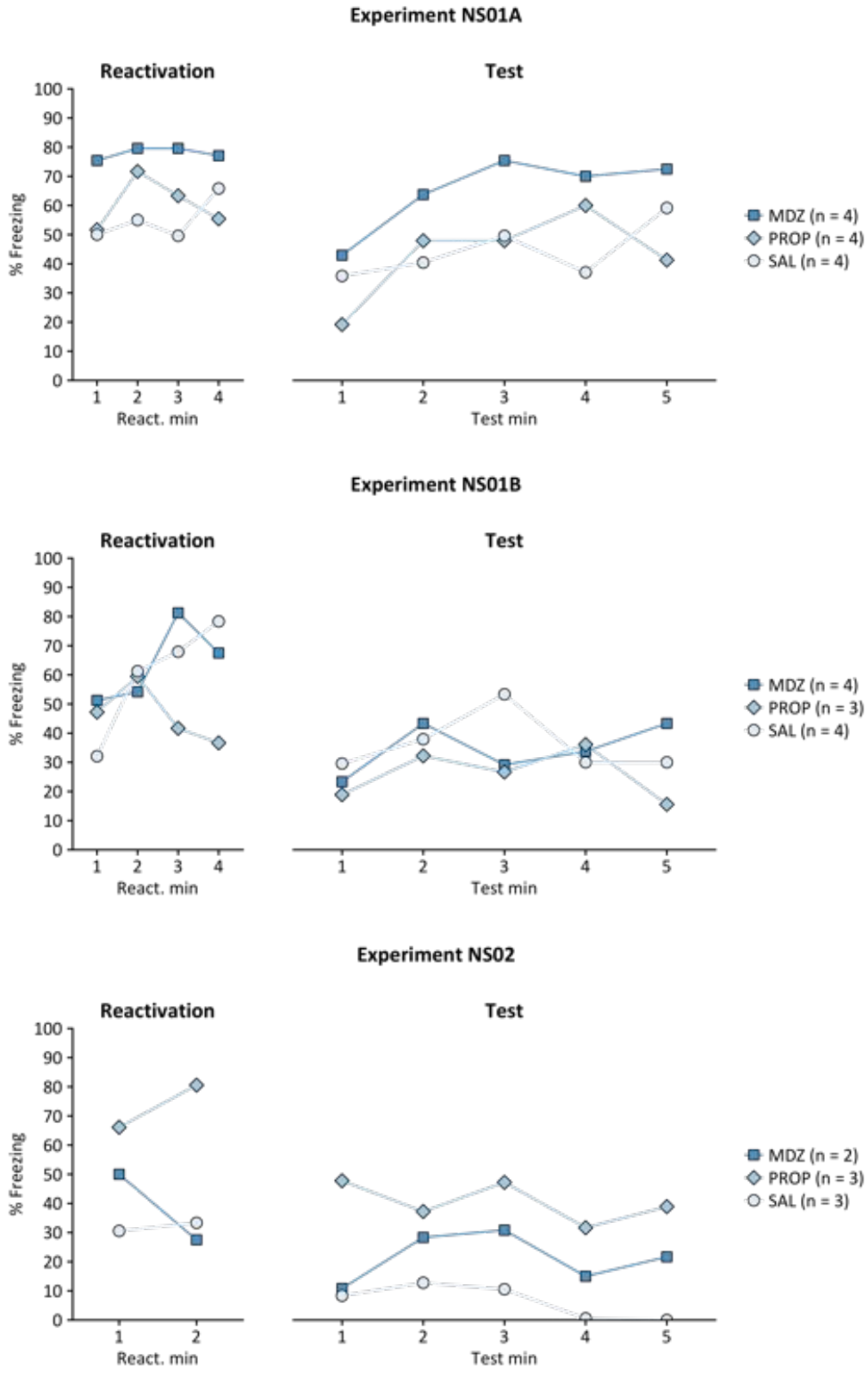

Training
CTX-US
React.
CTX
Test
CTX (5 min)

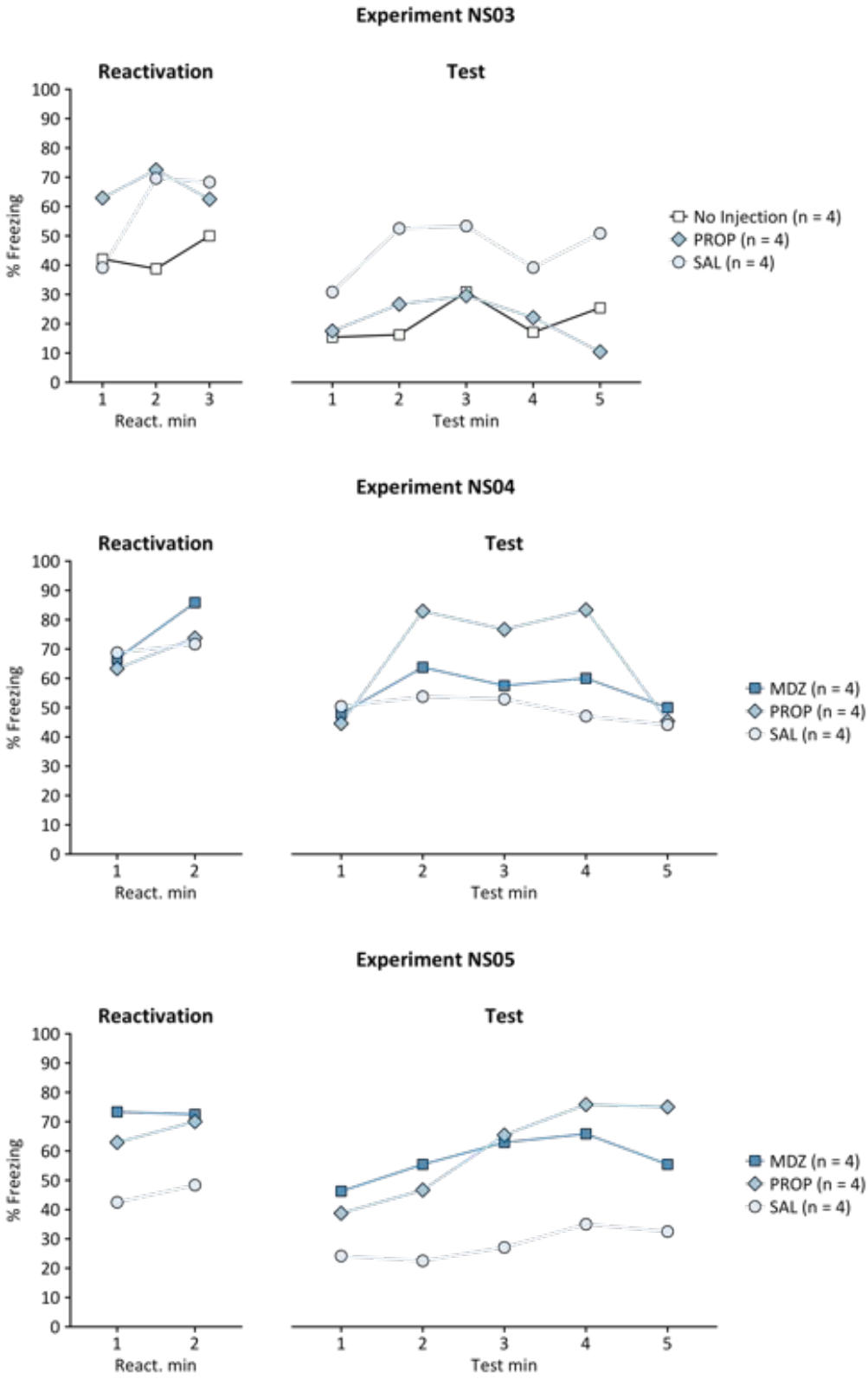

Experiment JA03
Reactivation
Test
% Freezing
React. min
Test min
MDZ (n = 2)
PROP (n = 4)
SAL (n = 4)

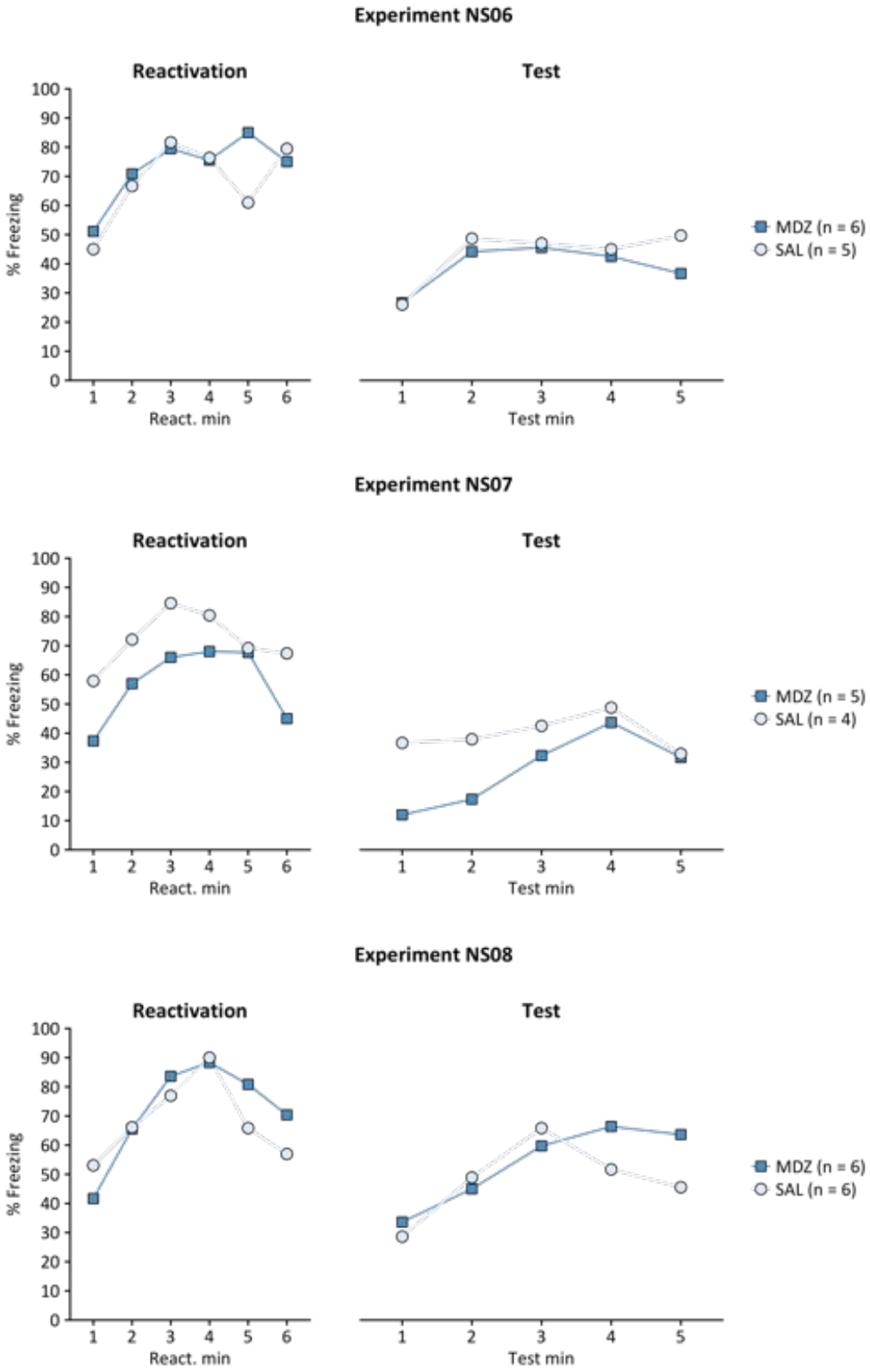

Experiment JA04
Reactivation
Test
% Freezing
React. min
Test min
MDZ (n = 3)
PROP (n = 4)
SAL (n = 4)

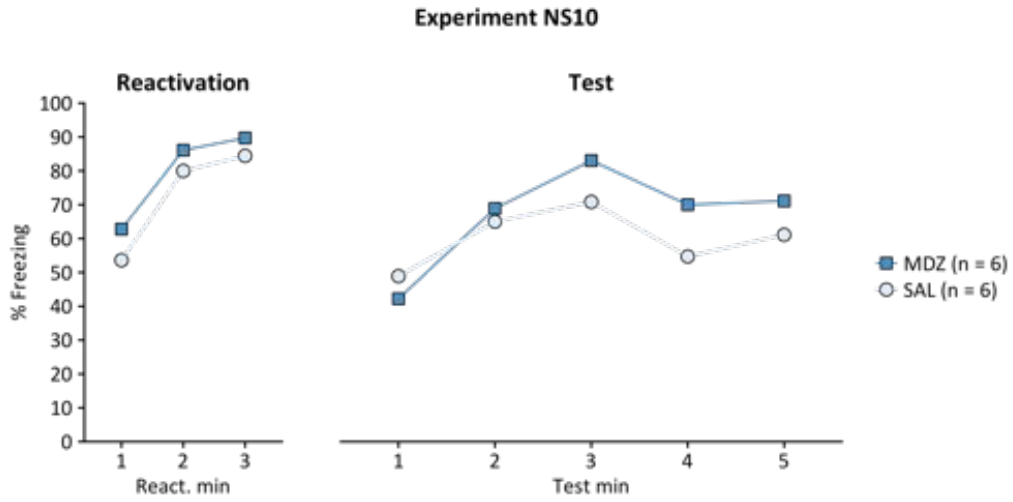

Experiment JA05
Reactivation
Test
% Freezing
React. min
Test min
MDZ (n = 4)
PROP (n = 4)
SAL (n = 3)

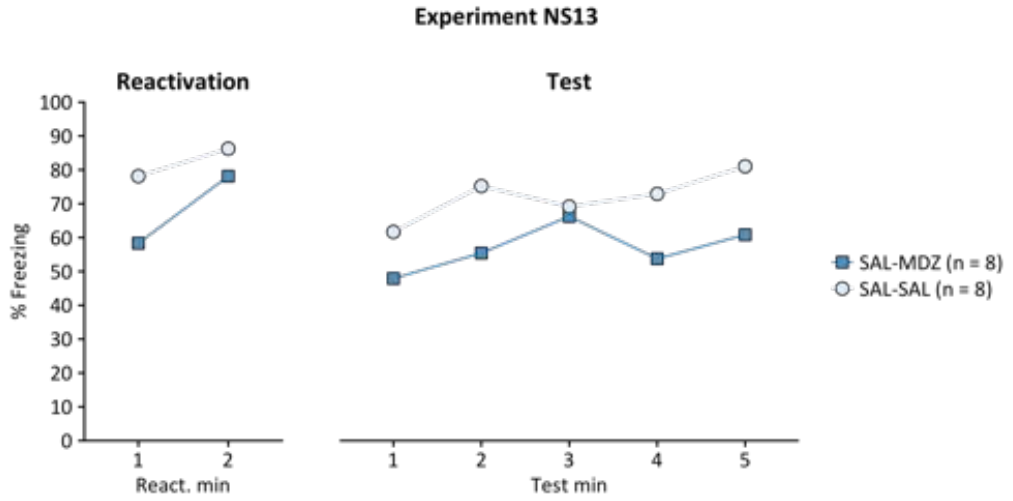

Experiment JA06
Reactivation
Test
% Freezing
React. min
Test min
MDZ (n = 3)
PROP (n = 2)
SAL (n = 3)
Experiment JA07
Reactivation
Test
% Freezing
React. min
Test min
MDZ (n = 4)
PROP (n = 3)
SAL (n = 4)
Experiment JA08
Reactivation
Test
% Freezing
React. min
Test min
MDZ (n = 5)
PROP (n = 6)
SAL (n = 5)

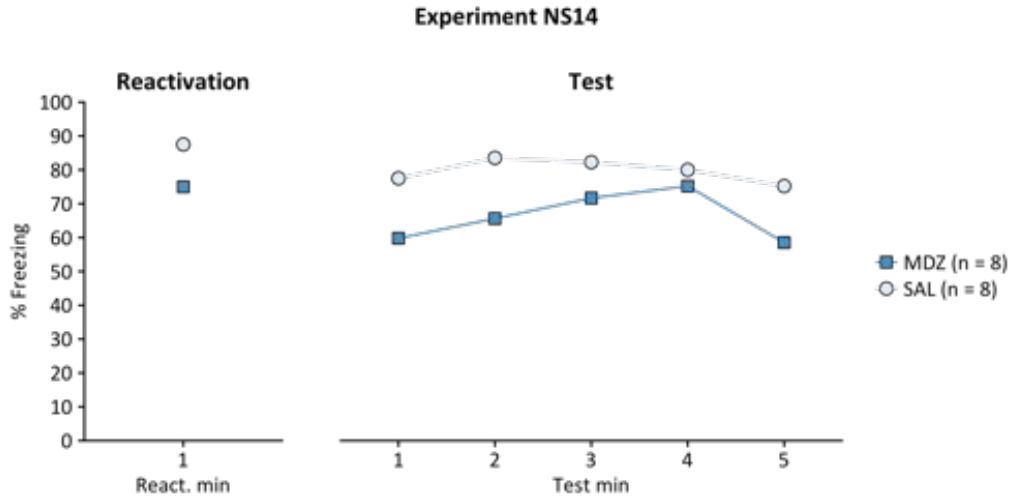

Experiment NS01A
Reactivation
Test
% Freezing
React. min
Test min
MDZ (n = 4)
PROP (n = 4)
SAL (n = 4)
Experiment NS01B
Reactivation
Test
% Freezing
React. min
Test min
MDZ (n = 4)
PROP (n = 3)
SAL (n = 4)
Experiment NS02
Reactivation
Test
% Freezing
React. min
Test min
MDZ (n = 2)
PROP (n = 3)
SAL (n = 3)


Experiment NS03
Reactivation
Test
% Freezing
React. min
Test min
No Injection (n = 4)
PROP (n = 4)
SAL (n = 4)
Experiment NS04
Reactivation
Test
% Freezing
React. min
Test min
MDZ (n = 4)
PROP (n = 4)
SAL (n = 4)
Experiment NS05
Reactivation
Test
% Freezing
React. min
Test min
MDZ (n = 4)
PROP (n = 4)
SAL (n = 4)


Experiment NS06
Reactivation
Test
% Freezing
React. min
Test min
MDZ (n = 6)
SAL (n = 5)
Experiment NS07
Reactivation
Test
% Freezing
React. min
Test min
MDZ (n = 5)
SAL (n = 4)
Experiment NS08
Reactivation
Test
% Freezing
React. min
Test min
MDZ (n = 6)
SAL (n = 6)


Experiment NS10
Reactivation
Test
% Freezing
React. min
Test min
MDZ (n = 6)
SAL (n = 6)


Experiment NS13
Reactivation
Test
% Freezing
React. min
Test min
SAL-MDZ (n = 8)
SAL-SAL (n = 8)


Experiment NS14
Reactivation
Test
% Freezing
React. min
Test min
MDZ (n = 8)
SAL (n = 8)

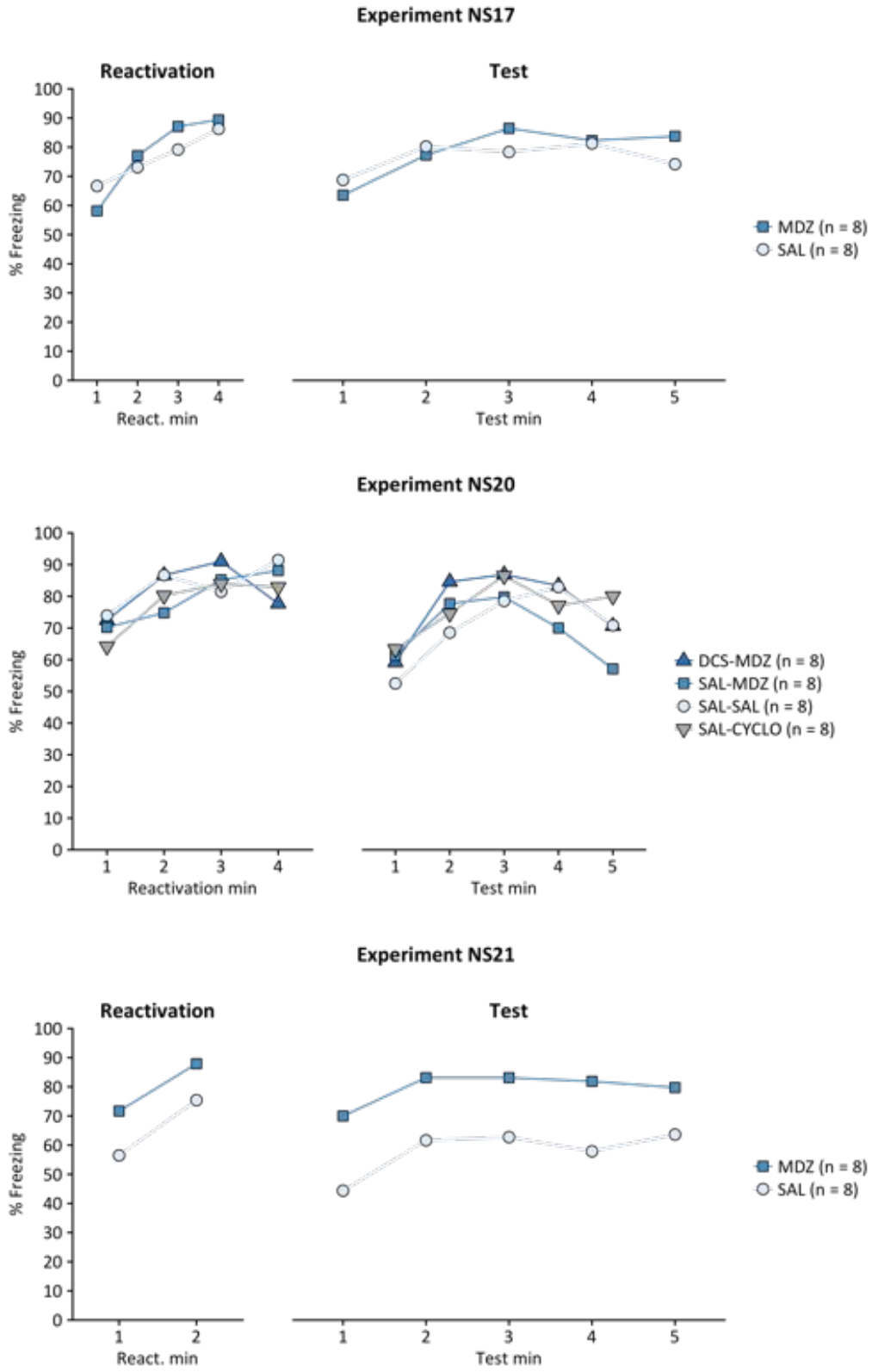
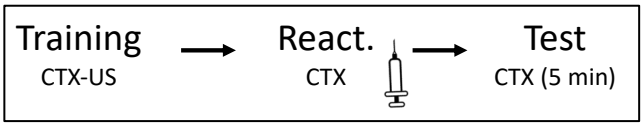
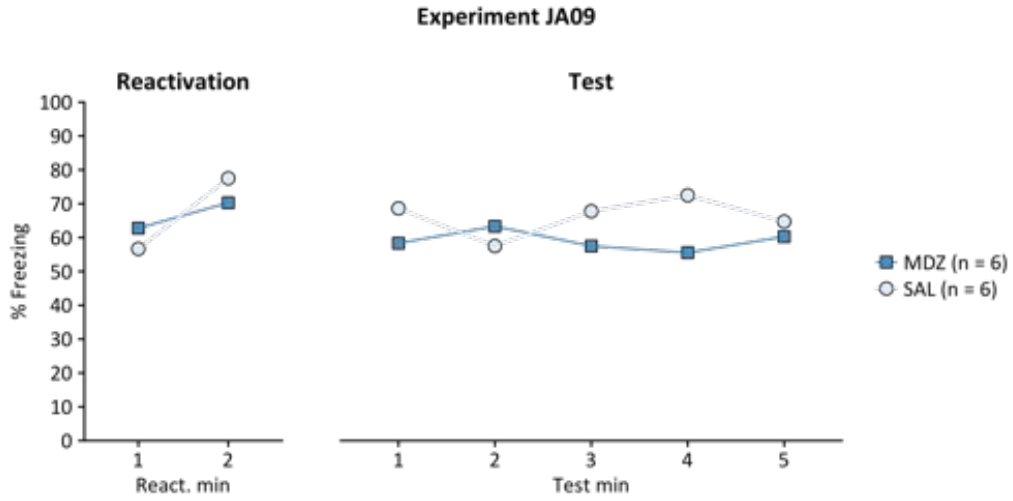
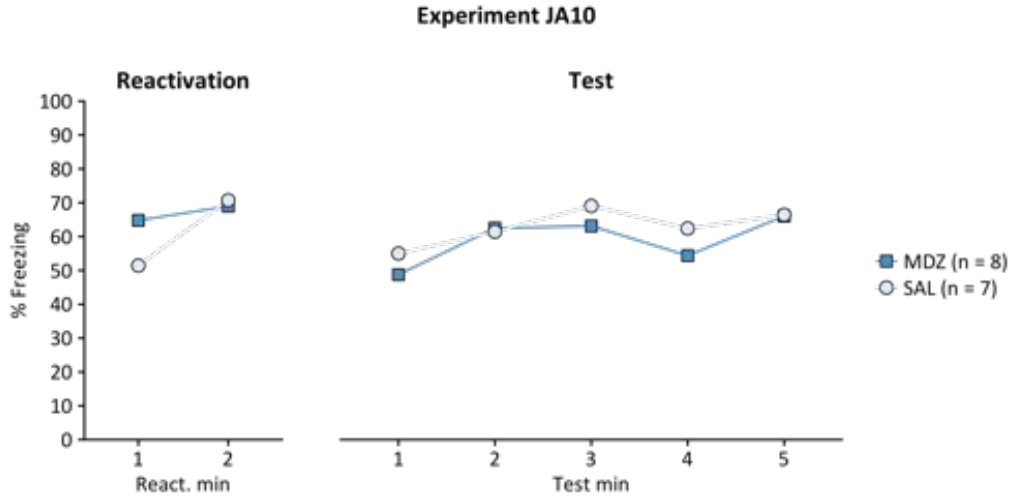
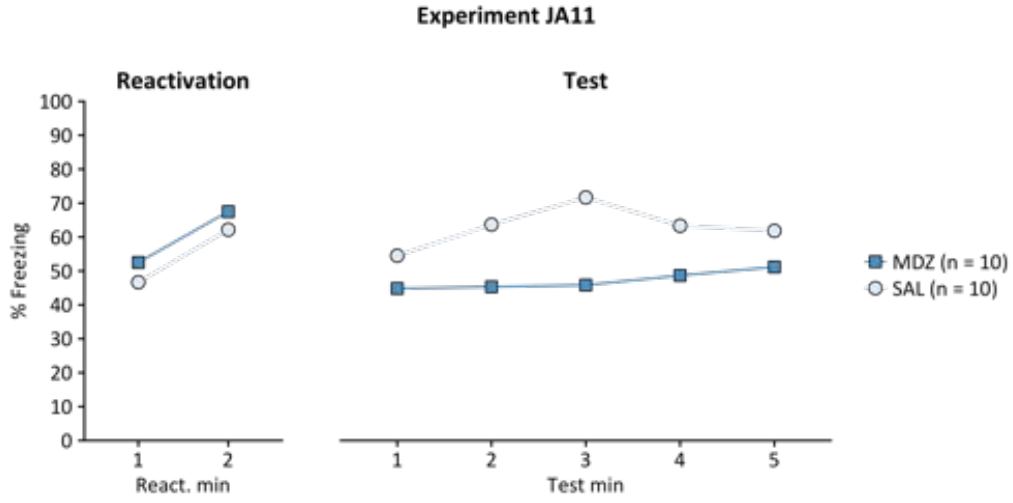


Figure D.1. Twenty-one failures to conceptually replicate post-reactivation amnesia induction for contextual fear memories. Group means are shown. Rats showing less than 25% freezing during the reactivation session were excluded. SAL = saline; MDZ = midazolam; PROP = propranolol; CYCLO = cycloheximide; DCS = D-cycloserine.


Training
CTX-US
React.
CTX
Test
CTX (5 min)


Experiment JA09
Reactivation
Test
% Freezing
React. min
Test min
MDZ (n = 6)
SAL (n = 6)


Experiment JA10
Reactivation
Test
% Freezing
React. min
Test min
MDZ (n = 8)
SAL (n = 7)


Experiment JA11
Reactivation
Test
% Freezing
React. min
Test min
MDZ (n = 10)
SAL (n = 10)

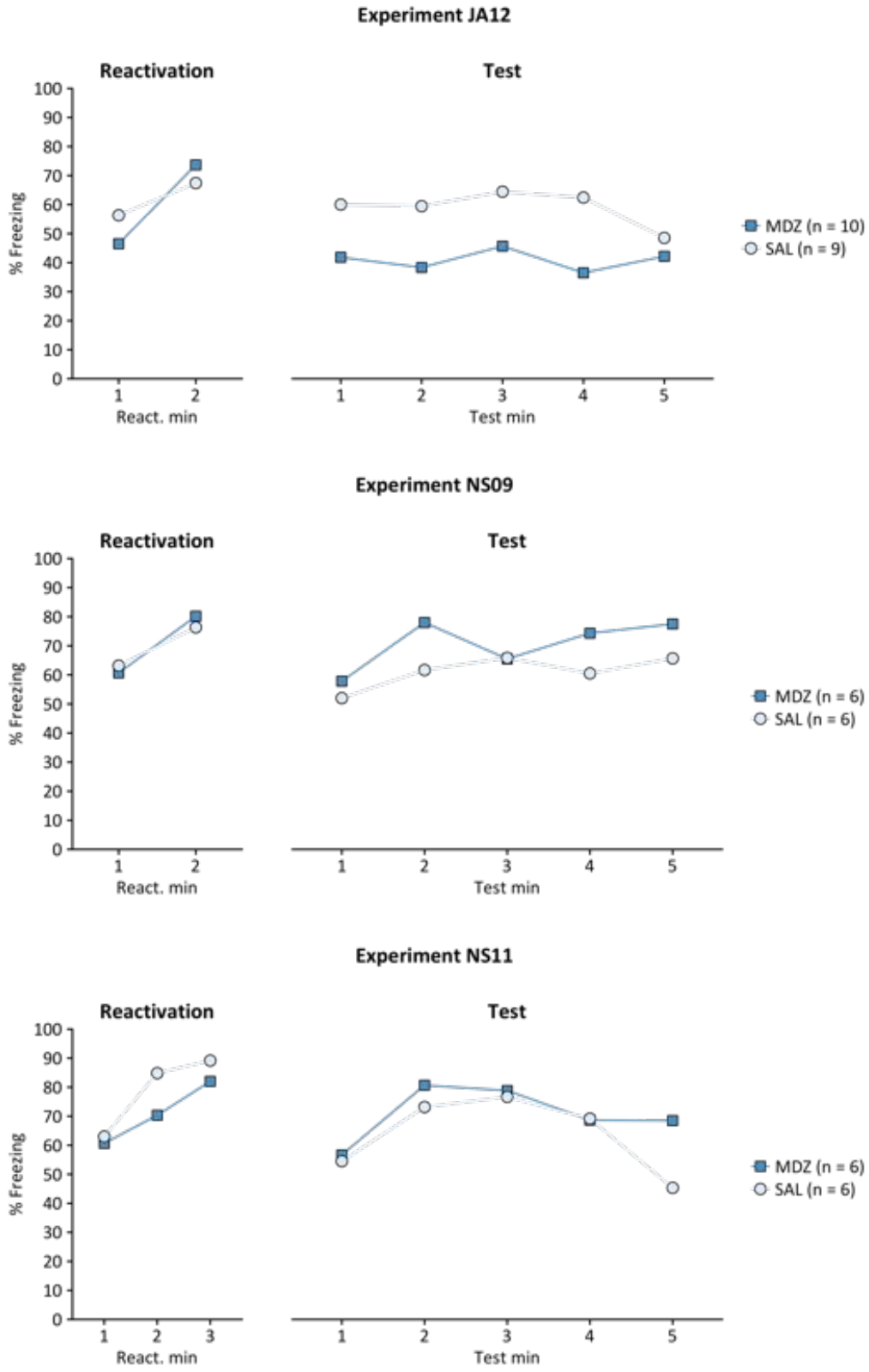


Figure C.2. Six attempts to exactly replicate post-reactivation amnesia induction for contextual fear memories. Group means are shown. Exact replication attempts following the methodology of Alfei et al. (2015) and Ferrer Monti et al. (2017) or Stern and colleagues (2012). Midazolam (MDZ) or saline (SAL) was administered systemically, immediately after contextual fear memory reactivation and fear memory retention was assessed 24 h later (Test, 5 min)

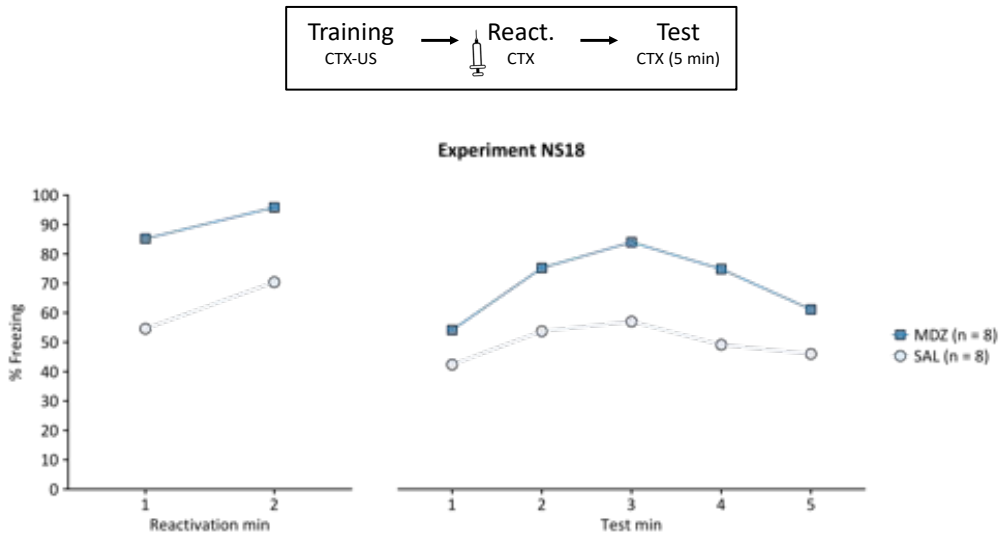


Figure C.3. No influence of pre-reactivation MDZ administration on fear memory retention. Group means are shown. Midazolam (MDZ) or saline (SAL) was administered 20 min before memory reactivation (React.) and fear memory retention was assessed 24 h later (Test, 5 min). MDZ enhanced freezing during the reactivation session due to an acute MDZ-induced change in locomotor activity. As a result, the change in % freezing from reactivation (under influence of MDZ) to test (drug-free state) in MDZ rats cannot be interpreted unambiguously.

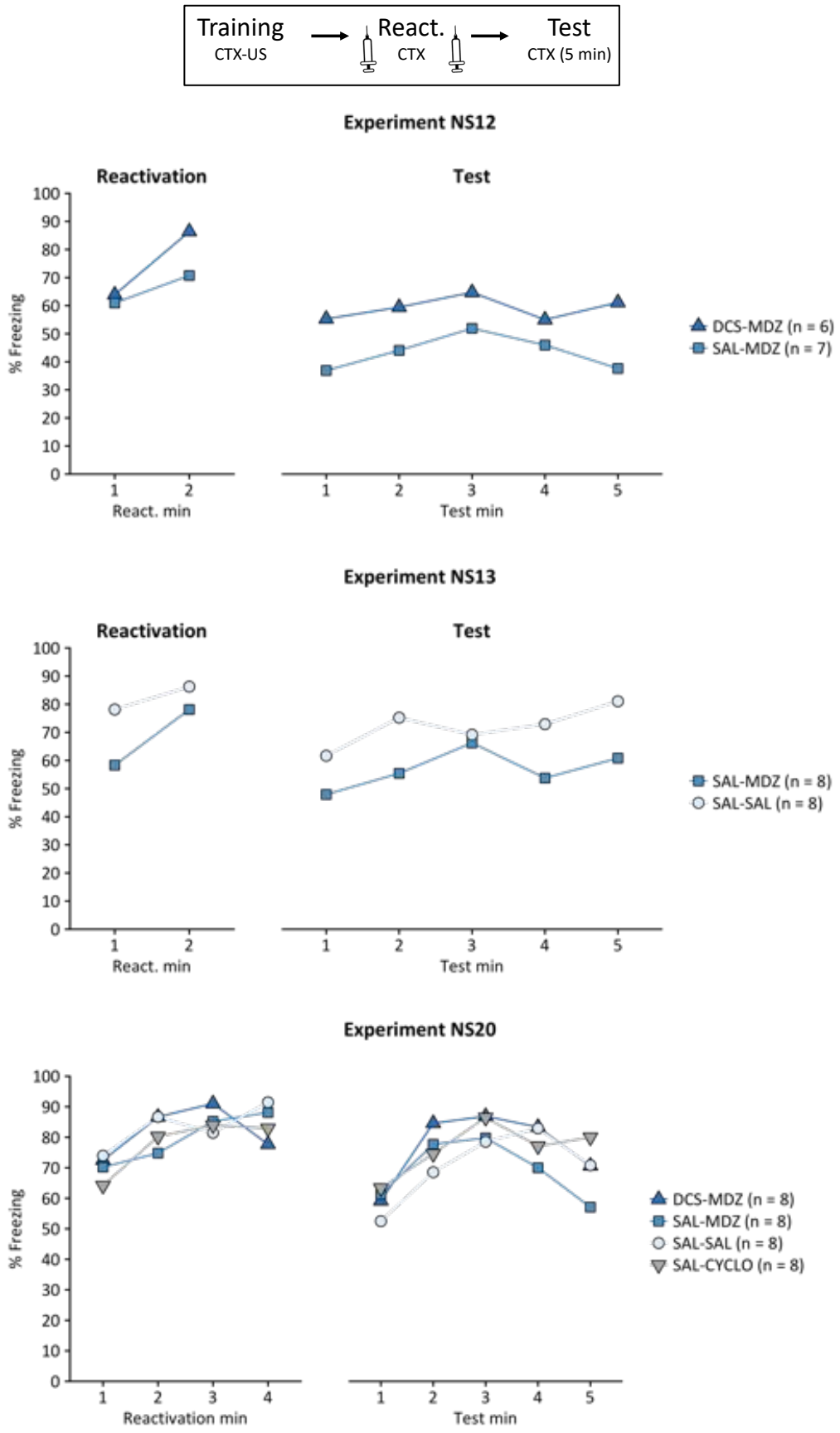


Figure C.4. No influence of pre-reactivation DCS administration on fear memory malleability. Group means are shown. D-cycloserine (DCS) or saline (SAL) was administered 30 min before memory reactivation, and midazolam (MDZ), cycloheximide (CYCLO) or SAL after reactivation. Fear memory retention was assessed 24 h later (Test, 5 min).

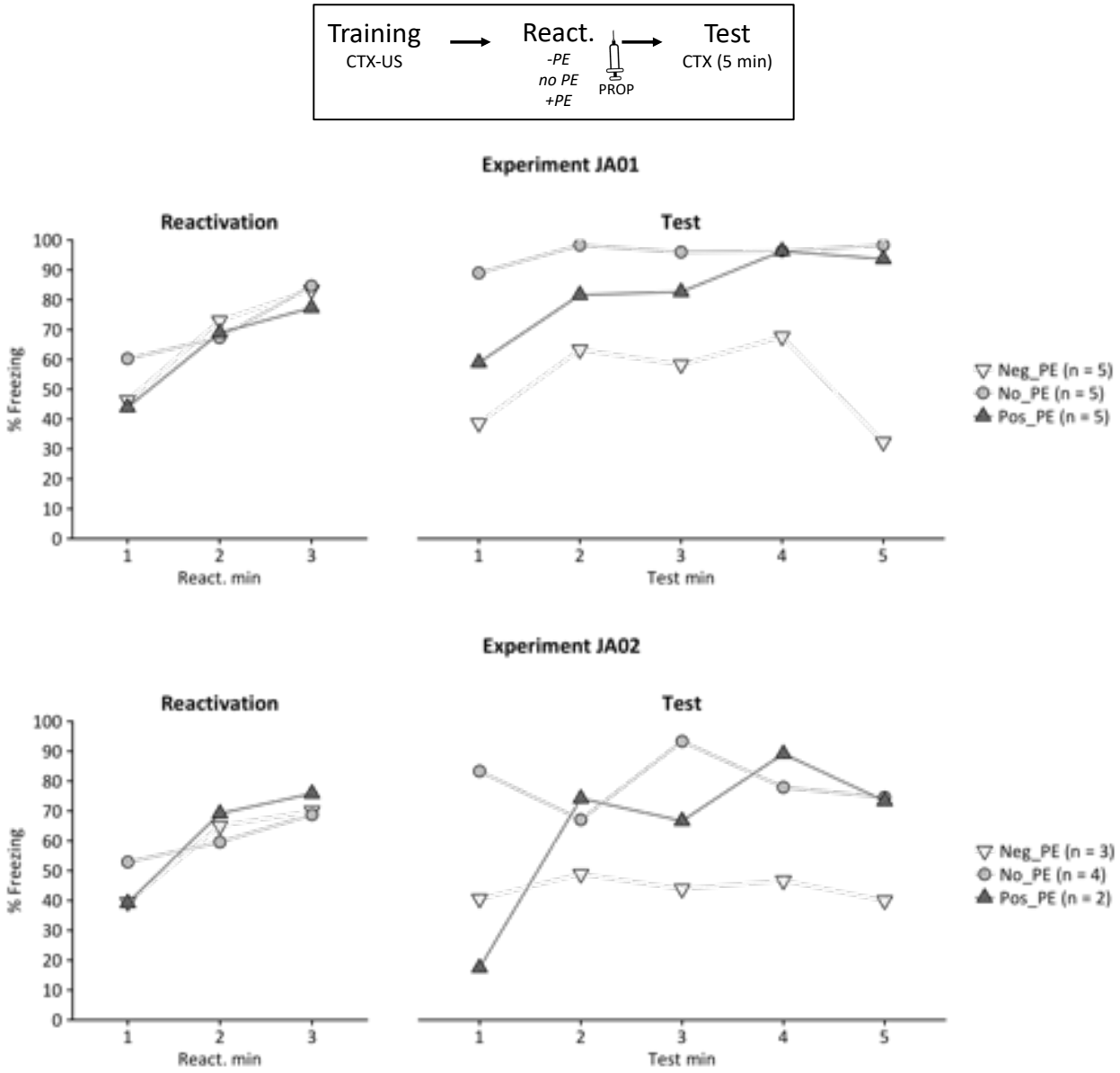


Figure C.5. Manipulating prediction error during reactivation did not allow for the induction of post-reactivation amnesia. Group means are shown. Propranolol (PROP) was administered systemically after memory reactivation (React.) and memory retention was assessed 24 h later (Test). During reactivation, animals were either retrained using the same parameters as during training (no prediction error, 'no PE') or using a higher shock intensity ('Positive PE'), or the shock was omitted ('Negative PE'). **(C)** During reactivation, animals were either retrained using the same parameters as during training ('no PE') or using more shocks (i.e., 2 shocks instead of 1 shock, 'positive PE'). In the third group, the shock was omitted ('negative PE').

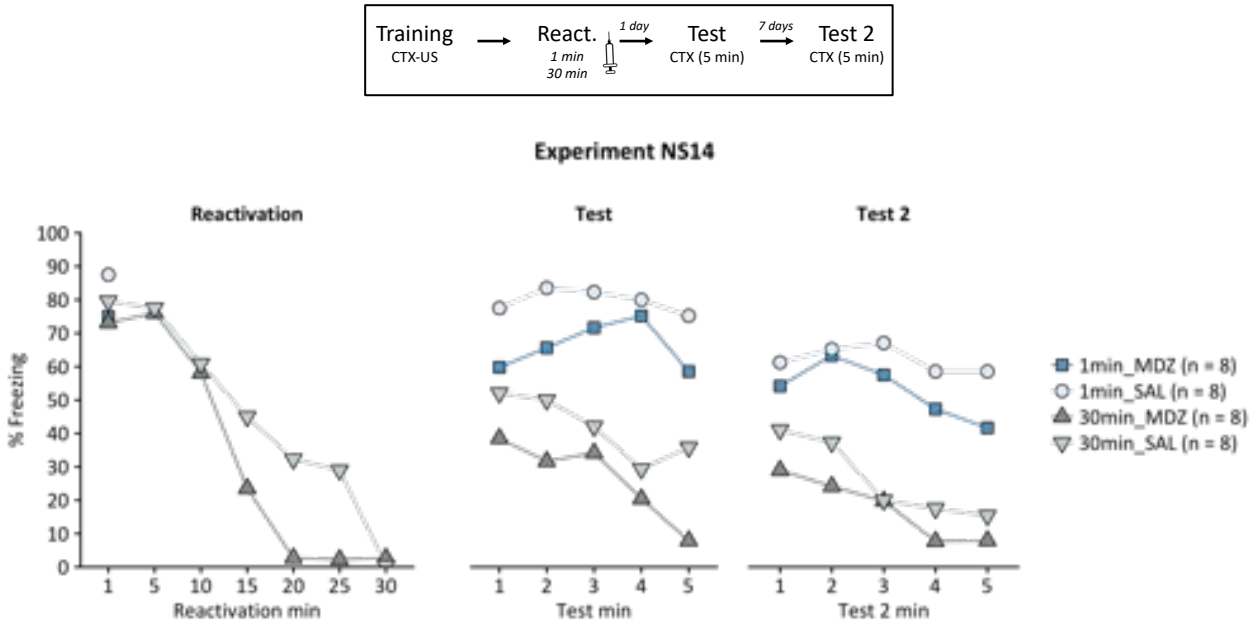


Figure C.6. MDZ administration after brief or long memory reactivation did not affect fear memory retention. Group means are shown. Midazolam (MDZ) or saline (SAL) was administered systemically after a brief (1 min) or long (30 min) memory reactivation session. Fear memory retention was assessed 1 (Test, 5 min) and 8 days later (Test 2, 5 min).

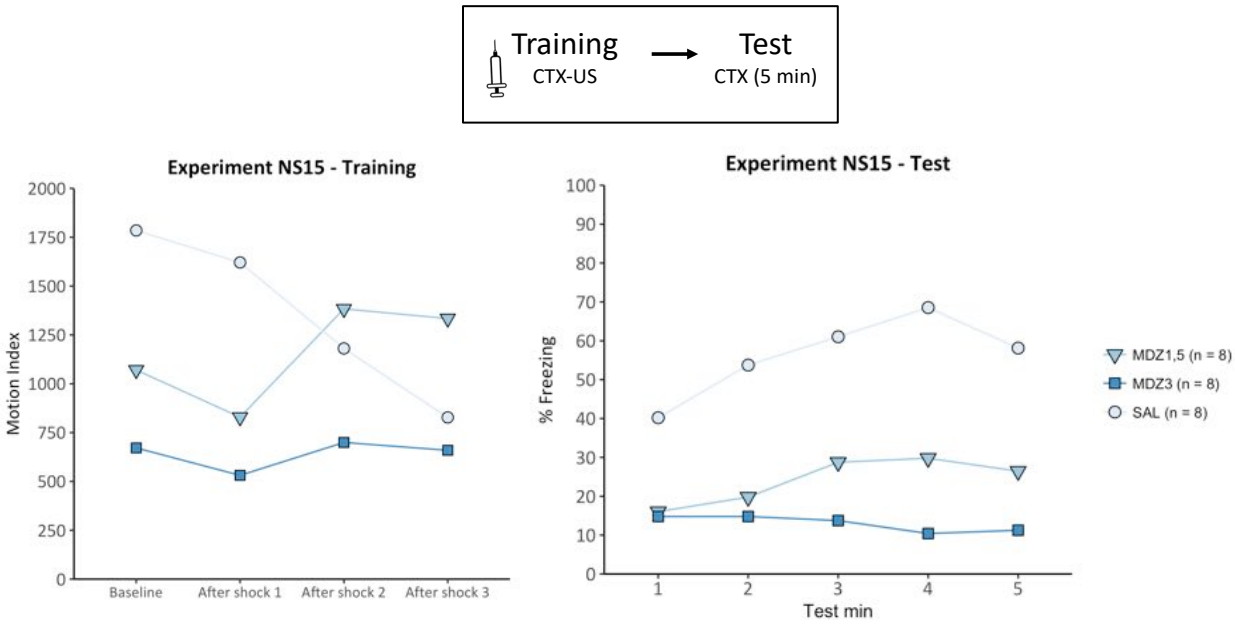


Figure D.7. MDZ administration before contextual fear conditioning acutely impairs locomotor activity and results in lower fear memory expression one day later. Group means are shown. Midazolam (MDZ) or saline (SAL) was administered systemically 20 min before contextual fear conditioning. MDZ induced a dose-dependent impairment in locomotor activity during the 3-min baseline period of the training session, as evidenced by a decrease in motion index (MedAssociates software). MDZ administration before learning impaired subsequent fear memory retention, as evidenced by lower freezing scores during a context exposure session one day later.