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Original Research

SUPPLEMENTAL ONLINE MATERIAL

Increased heroin intake and relapse vulnerability in intermittent relative to continuous self-administration: sex differences in rats

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Modeling brain levels of heroin and its metabolites. The following differential equations were used by Kinetica v.5.1 (Thermo Fisher Scientific Inc) to calculate the parameters to fit the ECF brain concentrations of heroin, 6-monoacetylmorphine, and morphine:

- One compartment:

$$Co = A\ \frac{Ka}{Ka{-}\propto}\left(e^{-\propto\, tl} - e^{-ka\, tl}\right)$$

- Two compartments:

$$Co = A\ \frac{Ka}{Ka{-}\propto}\left(e^{-\propto\, tl} - e^{-ka\, tl}\right) + B\ \ \frac{Ka/}{Ka-\beta}\ \left(e^{(-\beta\, tl)} - e^{(-ka\, tl)}\ \right)$$

- Three compartments:

$$Co = A\ \frac{Ka}{Ka{-}\propto}\left(e^{-\propto\, tl} - e^{-ka\, tl}\right) + B\ \ \frac{Ka}{Ka-\beta}\ \left(e^{(-\beta\, tl)} - e^{(-ka\, tl)}\right) + C\,\frac{Ka}{Ka-\gamma}\ \left(e^{(-\gamma\, tl)} - e^{(-ka\, tl)}\right.$$

Co is the concentration at time t; A, B and C are coefficients which describe the exponential functions, whereas α, β and γ are exponents which describe the shape of the measured concentration curve for each phase; Ka is the absorption rate; and tl=t-lag at time t.

From these "macro" constants, the PK parameters (**Supplementary Table 1**) are calculated as follows:

- One compartment:

$$Kel = \alpha$$

- Two compartments:

$$Co = A + B$$

$$K_{21} = \frac{A * \beta + B * \alpha}{Co}$$

$$K_{12} = \alpha + \beta - (K_{21} + Kel)$$

$$Kel = \frac{\alpha * \beta}{K_{21}}$$

- Three compartments:

$$Co = A + B + C$$

$$a = \alpha + \beta + \gamma$$

$$b = \frac{C * \alpha + B * \alpha + A * \gamma + B * \gamma + A * \beta + C * \beta}{-Co}$$

$$c = \frac{C * \alpha * \beta + B * \alpha * \gamma + A * \beta * \gamma}{Co}$$

$$K_{31} = \frac{-b - \sqrt{(b^2 - 4c)}}{2}$$

$$K_{21} = -b - K_{31}$$

$$Kel = \frac{\alpha * \beta * \gamma}{K_{21} - K_{31}}$$

$$K_{12} = \frac{(\beta * \gamma + \alpha * \beta + \alpha * \gamma - K_{21} * a - Kel * K_{31} + K_{21}^2)}{(K_{31} - K_{21})}$$

$$K_{13} = a - (Kel + K_{12} + K_{21} + K_{31})$$

These same equations are used during simulation to calculate the theoretical concentrations when the parameters are known. For administration of multiple doses, the software uses the superposition principle by independently computing the concentrations for each dose administered and afterwards adding the calculated concentrations for each time point of the session.

Supplementary Table 1. Pharmacokinetic parameters used in Gottås et al (Gottas et al., 2013) to fit the concentrations of heroin, 6-monoacetylmorphine (6-MAM), and morphine in the brain extracellular fluid after intravenous administration of 3 µmol (1.3 mg) heroin in the rat (Boix, Andersen & Morland, 2013). These same parameters were applied to the FitMultiMicroExtravascular model of the software program Kinetica v.5.1 (Thermo Fisher Scientific Inc., Waltham, MA, USA) to simulate the brain concentrations taking into account the times of the single infusions during the 10th self-administration training session.

Parameter	**Ka**	**lag**	**Volume**	**Kel**	**K12**	**K21**	**K13**	**K31**
Unit	min^{-1}	min	L	min^{-1}	min^{-1}	min^{-1}	min^{-1}	min^{-1}
Heroin[1]	0.749072	0.757626	0.401859	2.18171				
6-MAM[2]	1.00001	1.52153	0.417121	0.0695501	0.00802414	0.032289		
Morphine[3]	0.050732	1.22008	0.873335	0.0418547	0.114092	0.115256	0.0182552	0.012605

[1] One compartment extravascular model

[2] Two compartments extravascular model

[3] Three compartments extravascular model

Ka: Absorption rate constant from injection site. *Lag*: Time taken to appear in the brain following administration. *Kel*: Elimination rate constant from brain. *Volume*: Volume of distribution. *KXY*: Transfer rate constant between compartment X and Y (1 = measured compartment)

Supplementary Table 2. Statistical analysis (SPSS GLM repeated-measures module). Partial Eta^2 = proportion of explained variance.

Figure number	**Factor name**	**F-value**	***p*-value**	**Partial Eta^2**
Figure S2A SM7% intake (acquisition phase)	Session (within) Sex (between) Session X Sex interaction	$F_{2,240}$=52.272 $F_{1,120}$=54.315 $F_{2,240}$=7.782	**p=0.001*** **p=0.001*** **p=0.001***	0.300 0.308 0.060
Figure S2A SM7% intake (training phase)	Session (within) Sex (between) Session X Sex interaction	$F_{4,480}$=0.623 $F_{1,120}$=91.739 $F_{4,480}$=1.039	p=0.646 **p=0.001*** p=0.375	0.005 0.429 0.008
Figure S2B SM7% lever pressing (acquisition)	Session (within) Sex (between) Session X Sex interaction	$F_{2,120}$=8.477 $F_{1,120}$=20.317 $F_{2,120}$=5.873	**p=0.001*** **p=0.001*** **p=0.004***	0.065 0.143 0.046
Figure S2B SM7% lever pressing (training phase)	Session (within) Sex (between) Session X Sex interaction	$F_{4,120}$=6.975 $F_{1,120}$=2.768 $F_{4,120}$=2.725	**p=0.001*** p=0.099 **p=0.049***	0.054 0.022 0.022
Figure 2A Heroin intake (acquisition phase)	Session (within) Sex (between) Access (between) Session X Sex interaction Session X Access interaction Session X Sex X Access interaction	$F_{2,236}$=0.739 $F_{2,118}$=0.000 $F_{2,118}$=1.017 $F_{2,236}$=0.904 $F_{2,236}$=1.724 $F_{2,236}$=0.410	p=0.479 p=0.996 p=0.315 p=0.406 p=0.181 p=0.664	0.006 0.000 0.008 0.007 0.014 0.003
Total heroin intake (acquisition phase)	Access (between) Sex (between) Access X Sex	$F_{3,120}$=1.017 $F_{3,120}$=0.000 $F_{3,120}$=0.030	p=0.315 p=0.996 p=0.862	0.008 0.000 0.000
Figure 2A Heroin intake (training phase)	Session (within) Sex (between) Access (between) Session X Sex interaction Session X Access interaction Session X Sex X Access interaction	$F_{9,1080}$=36.061 $F_{9,120}$=0.359 $F_{9,120}$=3.960 $F_{9,1080}$=1.462 $F_{9,1080}$=1.273 $F_{9,1080}$=3.673	**p=0.001*** p=0.550 **p=0.049*** p=0.229 p=0.262 p=0.058	0.231 0.003 0.032 0.012 0.010 0.030
Figure 2B Heroin frequency of intake (acquisition phase)	Session (within) Sex (between) Access (between) Session X Sex interaction Session X Access interaction Session X Sex X Access interaction	$F_{2,240}$=0.739 $F_{1,120}$=0.000 $F_{1,120}$=1.017 $F_{2,240}$=0.904 $F_{2,240}$=1.724 $F_{2,240}$=0.410	p=0.436 p=0.996 p=0.315 p=0.344 p=0.181 p=0.594	0.006 0.0001 0.008 0.007 0.014 0.003
Figure 2B Heroin frequency of intake (training phase)	Session (within) Sex (between) Access (between) Session X Sex interaction Session X Access interaction Session X Sex X Access interaction	$F_{9,1080}$=30.322 $F_{1,120}$=0.113 $F_{1,120}$=156.573 $F_{9,1080}$=1.108 $F_{9,1080}$=17.035 $F_{9,1080}$=2.018	**p=0.001*** p=0.737 **p=0.001*** p=0.350 **p=0.001*** p=0.099	0.202 0.001 0.566 0.009 0.124 0.017
Figure 2C Heroin active lever pressing (acquisition phase)	Session (within) Sex (between) Access (between)	$F_{2,236}$=0.851 $F_{1,118}$=0.992 $F_{1,118}$=3.257	p=0.375 p=0.372 p=0.074	0.017 0.008 0.026

	Session X Sex interaction	$F_{2,236}=0.439$	p=0.645	0.004
	Session X Access interaction	$F_{2,236}=4.262$	**p=0.041***	0.034
	Session X Sex X Access interaction	$F_{2,236}=0.889$	p=0.412	0.007
Figure 2C Heroin active lever pressing (training phase)	Session (within)	$F_{9,1035}=24.656$	**p=0.001***	0.174
	Sex (between)	$F_{1,115}=0.013$	p=0.911	0.000
	Access (between)	$F_{1,115}=0.490$	p=0.485	0.004
	Session X Sex interaction	$F_{9,1035}=2.453$	**p=0.008***	0.021
	Session X Access interaction	$F_{9,1035}=0.675$	p=0.594	0.006
	Session X Sex X Access interaction	$F_{9,1035}=2.705$	**p=0.030***	0.023
Heroin inactive lever pressing (acquisition phase)	Session (within)	$F_{2,236}=0.372$	p=0.689	0.003
	Sex (between)	$F_{1,118}=1.532$	p=0.218	0.013
	Access (between)	$F_{1,118}=0.118$	p=0.732	0.001
	Session X Sex interaction	$F_{2,236}=0.048$	p=0.954	0.0001
	Session X Access interaction	$F_{2,236}=0.490$	p=0.613	0.004
	Session X Sex X Access interaction	$F_{2,236}=0.161$	p=0.852	0.001
Heroin inactive lever pressing (training phase)	Session (within)	$F_{9,1062}=4.971$	**p=0.001***	0.004
	Sex (between)	$F_{1,118}=0.679$	p=0.412	0.006
	Access (between)	$F_{1,118}=0.535$	p=0.466	0.005
	Session X Sex interaction	$F_{9,1062}=0.692$	p=0.717	0.006
	Session X Access interaction	$F_{9,1062}=0.950$	p=0.480	0.008
	Session X Sex X Access interaction	$F_{9,1062}=0.498$	p=0.877	0.004
Inter-infusion intervals (continuous access)	Sex	$F_{1,58}=0.439$	p=0.510	0.008
Figure 3A Cumulative infusions (10th session)	Continuous access			
	Sex (between)	$F_{1,63}=1.875$	p=0.085	1.000
	Intermittent access			
	Sex (between)	$F_{1,60}= 1.736$	p=0.067	0.619
Figure 3B 1st minute	Session (within)	$F_{9,59}=13.256$	**p=0.001***	0.183
	Sex (between)	$F_{1,59}=0.194$	p=0.661	0.003
	Session X Sex interaction	$F_{9,59}=1.571$	p=0.215	0.026
Figure 3B 2nd minute	Session (within)	$F_{9,59}=1.062$	p=0.307	0.018
	Sex (between)	$F_{1,59}=0.007$	p=0.936	0.000
	Session X Sex interaction	$F_{9,59}=1.141$	p=0.290	0.019
Figure 3B 3rd minute	Session (within)	$F_{9,59}=1.151$	p=0.223	0.025
	Sex (between)	$F_{1,59}=0.014$	p=0.905	0.000
	Session X Sex interaction	$F_{9,59}=0.349$	p=0.557	0.006
Figure 3B 4th minute	Session (within)	$F_{9,59}=2.347$	p=0.131	0.038
	Sex (between)	$F_{1,59}=0.607$	p=0.439	0.010
	Session X Sex interaction	$F_{9,59}=0.437$	p=0.511	0.007
Figure 3B 5th minute	Session (within)	$F_{9,59}=1.005$	p=0.410	0.017
	Sex (between)	$F_{1,59}=0.643$	p=0.426	0.011
	Session X Sex interaction	$F_{9,59}=0.698$	p=0.711	0.012
Discrete-choice tests Preference score	Session (within)	$F_{1,55}=0.066$	p=0.798	0.0001
	Sex (between)	$F_{1,55}=2.488$	p=0.120	0.043
	Access (Between)	$F_{1,55}=0.822$	p=0.386	0.015
	Session X Sex interaction	$F_{1,55}=0.013$	p=0.191	0.003
	Session X Access interaction	$F_{1,55}=2.452$	p=0.123	0.043

	Session X Sex X Access interaction	$F_{1,55}$=0.031	p=0.860	0.0001
Figure 5 Voluntary abstinence Preference score	Session (within) Sex (between) Access (between) Session X Sex interaction Session X Access interaction Session X Sex X Access interaction	$F_{17,765}$=4.010 $F_{1,45}$=0.156 $F_{1,45}$=3.630 $F_{17,765}$=0.949 $F_{17,765}$=0.502 $F_{17,765}$=0.686	**p=0.001*** p=0.695 p=0.054 p=0.515 p=0.953 p=0.818	0.082 0.003 0.032 0.021 0.011 0.015
Figure 6A (left) Relapse (incubation) test	Abstinence day (within) Access (between) Sex (between) Abstinence condition (between) Abstinence day X Sex interaction Abstinence day X Access interaction Abstinence day X Abstinence condition interaction Abstinence day X Sex X Access interaction Abstinence day X Sex X Abstinence condition interaction Abstinence day X Access X Abstinence condition interaction Abstinence day X Sex X Access X Abstinence condition interaction	$F_{1,94}$=14.699 $F_{1,94}$=5.895 $F_{1,94}$=3.105 $F_{1,94}$=2.841 $F_{1,94}$=0.003 $F_{1,94}$=7.239 $F_{1,94}$=8.826 $F_{1,94}$=0.544 $F_{1,94}$=0.126 $F_{1,94}$=0.232 $F_{1,94}$=0.136	**p=0.001*** **p=0.017*** p=0.081 p=0.095 p=0.991 **p=0.009*** **p=0.004*** p=0.462 p=0.606 p=0.631 p=0.714	0.135 0.059 0.032 0.029 0.0001 0.072 0.086 0.006 0.003 0.002 0.001
Figure 6A (left) Relapse (incubation) test (Day 1)	Sex (between) Access (between) Abstinence condition (between)	$F_{1,94}$=2.325 $F_{1,94}$=12.056 $F_{1,94}$=0.006	p=0.131 **p=0.001*** p=0.941	0.024 0.114 0.000
Figure 6A (center) Relapse (incubation) test (continuous access)	Abstinence day (within) Sex (between) Abstinence condition (between) Abstinence day X Sex interaction Abstinence day X Abstinence condition interaction Abstinence day X Sex X Abstinence condition interaction	$F_{1,44}$=24.872 $F_{1,44}$=0.149 $F_{1,44}$=1.829 $F_{1,44}$=0.329 $F_{1,44}$=6.985 $F_{1,44}$=0.0001	**p=0.001*** p=0.702 p=0.183 p=0.569 **p=0.011*** p=0.992	0.361 0.003 0.040 0.007 0.137 0.0001
Figure 6A (right) Relapse (incubation) test (intermittent access)	Abstinence day (within) Sex (between) Abstinence condition (between) Abstinence day X Sex interaction Abstinence day X Abstinence condition interaction Abstinence day X Sex X Abstinence condition interaction	$F_{1,50}$=0.586 $F_{1,50}$=4.245 $F_{1,50}$=1.138 $F_{1,50}$=0.240 $F_{1,50}$=2.826 $F_{1,50}$=0.238	p=0.447 **p=0.046*** p=0.291 p=0.626 p=0.099 p=0.628	0.012 0.078 0.022 0.005 0.054 0.005

Figure 6B Time course relapse test (Day 1)	Minute (within) Sex (between) Access (between) Abstinence condition (between) Sex X Access interaction Sex X Abstinence condition interaction Access X Abstinence condition interaction Sex X Access X Abstinence condition interaction	$F_{2,94}$=74.953 $F_{2,94}$=2.209 $F_{2,94}$=12.416 $F_{2,94}$=0.001 $F_{2,94}$=2.535 $F_{2,94}$=0.131 $F_{2,94}$=1.779 $F_{2,94}$=0.918	**p=0.001*** p=0.141 **p=0.001*** p=0.082 p=0.082 p=0.854 p=0.176 p=0.401	0.444 0.023 0.117 0.026 0.026 0.001 0.019 0.010
Figure 6C Time course relapse test (Day 21)	Minute (within) Sex (between) Access (between) Abstinence condition (between) Sex X Access interaction Sex X Abstinence condition interaction Access X Abstinence condition interaction Sex X Access X Abstinence condition interaction	$F_{2,94}$=34.607 $F_{2,94}$=1.719 $F_{2,94}$=0.550 $F_{2,94}$=7.167 $F_{2,94}$=0.383 $F_{2,94}$=2.809 $F_{2,94}$=0.988 $F_{2,94}$=0.933	**p=0.001*** p=0.193 p=0.550 **p=0.009*** p=0.682 p=0.063 p=0.374 p=0.395	0.269 0.018 0.460 0.071 0.004 0.029 0.010 0.010
Figure S3 Heroin intake: estrous cycle (5 days)	Continuous access Intermittent access	t=0.0187 t=0.1857	p=0.985 p=0.853	df=44 df=44
Figure 7A Relapse (incubation) test: estrous cycle (Abstinence day 1)	Access (between) Cycle (between) Access X Cycle interaction	$F_{1,44}$=5.874 $F_{1,44}$=0.043 $F_{1,44}$=0.015	**p=0.013*** p=0.837 p=0.903	0.118 0.001 0.0001

Figure S1

Apparatus

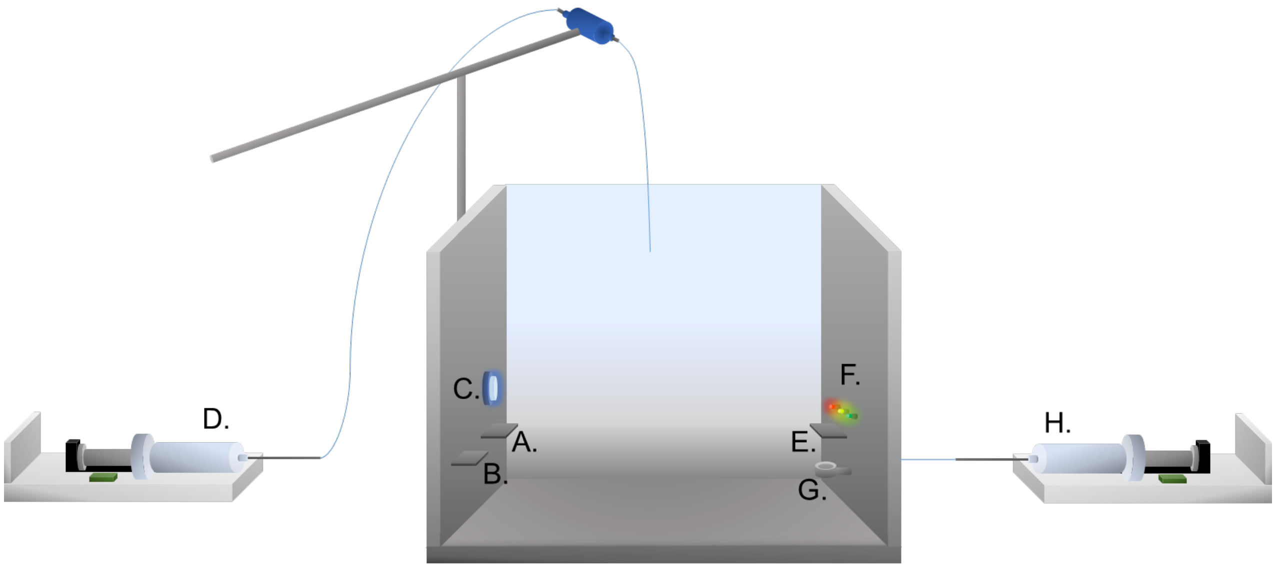

Figure S1. *Apparatus.* (**A**) Active heroin lever. (**B**) Inactive heroin lever. (**C**) White-light cue. (**D**) Heroin pump. (**E**) Sucrose+Maltodextrine 7% lever. (**F**) Three-light cue. (**G**) Receptacle. (**H**) Sucrose+Maltodextrine 7% pump.

Figure S2

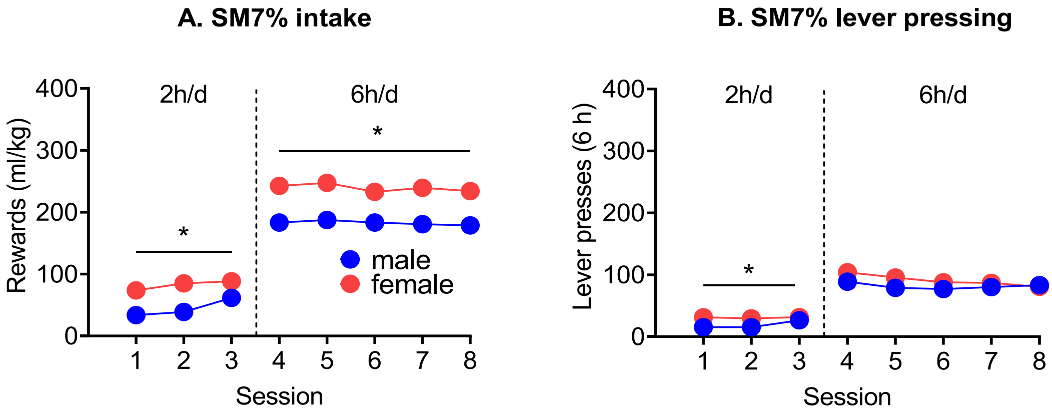

Figure S2. *Sucrose + Maltodextrin 7% self-administration training (SM7%).* (**A**) <u>SM7% intake.</u> Mean±SEM number of SM7% rewards earned (1 ml infusion) during sessions. (**B**) <u>SM7% lever pressing</u>. Mean±SEM number of lever presses during sessions. *Different from males, $p<0.05$ (males n=53 / females n=71).

Figure S3

Heroin intake: estrous cycle

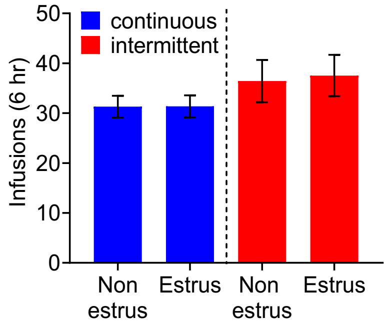

Figure S3. *Effect of estrous cycle on continuous or intermittent heroin self-administration in female rats.* Heroin intake: estrous cycle. Mean±SEM of the average of infusions earned during non-estrus and estrus phase in the last five days of heroin self-administration (n=48).

References

Boix F, Andersen JM, & Morland J (2013). Pharmacokinetic modeling of subcutaneous heroin and its metabolites in blood and brain of mice. Addict Biol 18**:** 1-7.

Gottas A, Oiestad EL, Boix F, Vindenes V, Ripel A, Thaulow CH, *et al.* (2013). Levels of heroin and its metabolites in blood and brain extracellular fluid after i.v. heroin administration to freely moving rats. Br J Pharmacol 170**:** 546-556.