

1.1 Introduction: re-design of odour delivery system

We are re-designing the odour delivery system to deal with the problems we've been having. The main problem is the contamination of the odour 'ring' leading to it becoming permanently smelly. There is evidence that the previous odour presentation hangs around in the system and contaminates the current presentation.

We will be addressing the issue by re-building the whole system. The following are planned:

- Use proper MFCs to regulate flow rates.
- Use solenoid valves on both input and output sides. We will try out the N-Research zero dead-space linear valve arrays.
- See if we can get away without a final valve by placing this arrangement near the fly.

1.2 Things I've Learned

Most of this document is a 'lab-book' describing my attempts to make the odour delivery system behave properly. In the course of doing this, I've obviously learned a lot. This section summarises the most interesting information so one doesn't have to comb through the whole document to learn stuff.

- One can get surprisingly good kinetics even without a final valve (Fig. 1.1, p. 2). In that Figure we're using 1/8" tubing and pure ethanol. The problem is that the head-space needs to be equilibrated to attain a consistent response (see how good it *can* look: Fig. 1.13, p. 12) and this requires a final valve.
- I found that the blue check valves (those that whistle) require at least about 200 ml/min to open (see p. 3). If flow is less than that, they may not open (e.g. see Fig. 1.1)

1.2.1 23rd March 2010

Today I strip the valves off the old system. Clean Teflon pieces and chuck the tubing. I checked the function of each solenoid and discovered that about 8 valves on the side nearer to the microscope are delivering very low quantities of air. We will ignore this side for now. Connect up only vials 11:2:29 in series. Fill each vial with 5ml of ethanol. Vial 29 is empty. All others are disconnected. I am using the narrow (pink) needles for both input and output.

We record the signal with and without check valves. There is no final valve so the PID is on the end of the tube coming away from the vials. Notice that there is odour coming out of clean air with the check valve. But we have just cleaned them with ethanol.

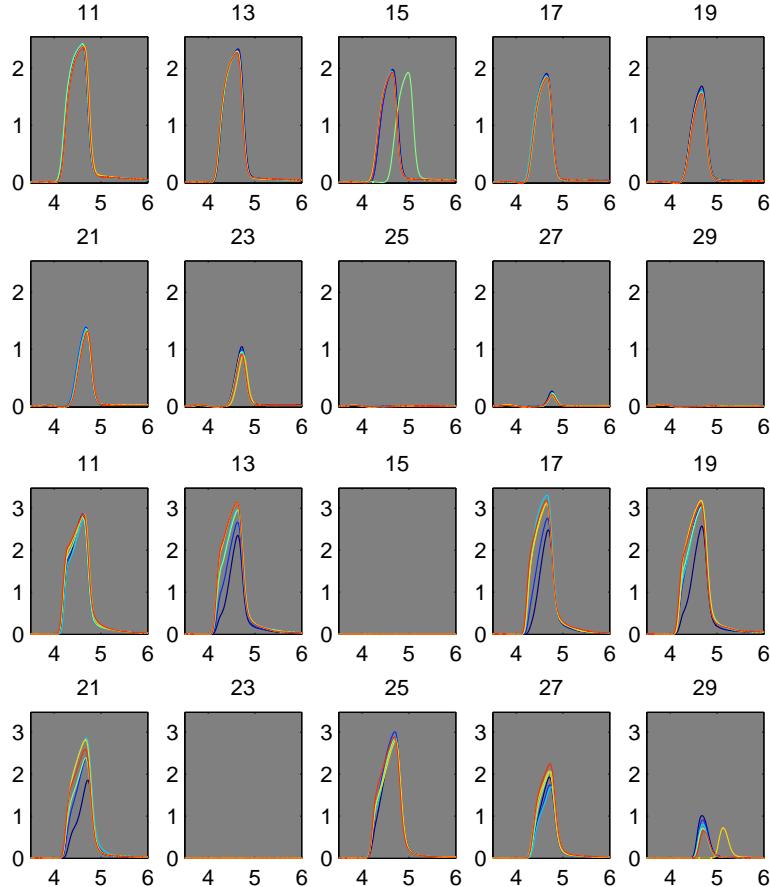


Figure 1.1: Upper two rows is `params_100323_163943`, with check valves. Lower two rows is `params_100323_173117` with check valves. I later discovered that the response failures were due to sticking check-valves.

1.2.2 24th March 2010

Today we try it again. The clean air signal has vastly decreased. I realised that the check valves were sticking. Passing air through at 800 ml/min does unstick them. However, just playing with the machine it seems that the PID traces are rather non-stationary. Actually, when I run it for 6 reps

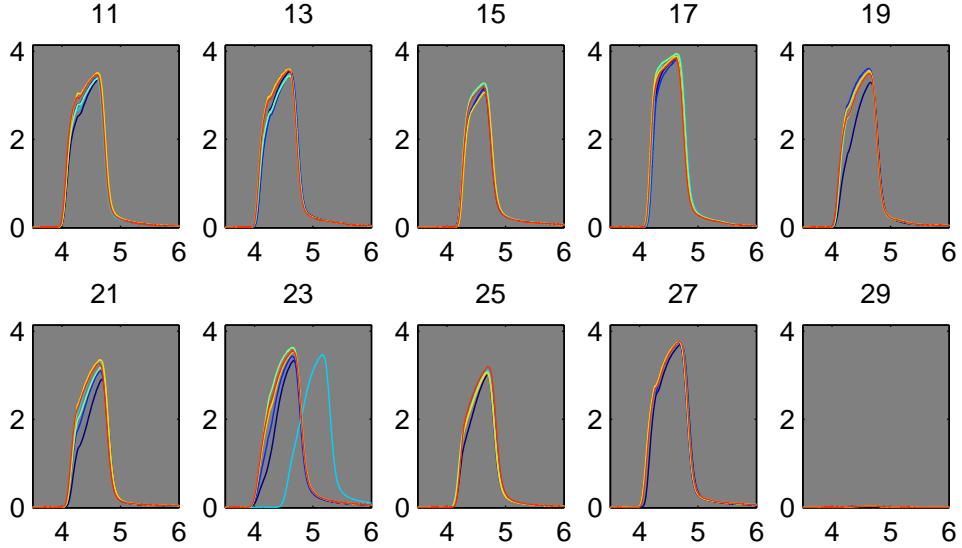


Figure 1.2: params_100324_121954:

it looks pretty good. The only issue is that sometimes the odour presentation is delayed by 500 ms (happened 1/60 in this case). I think this is due to the flow controller signal being delayed. There is some non-stationarity and I generally notice a trend for larger responses later on in time.

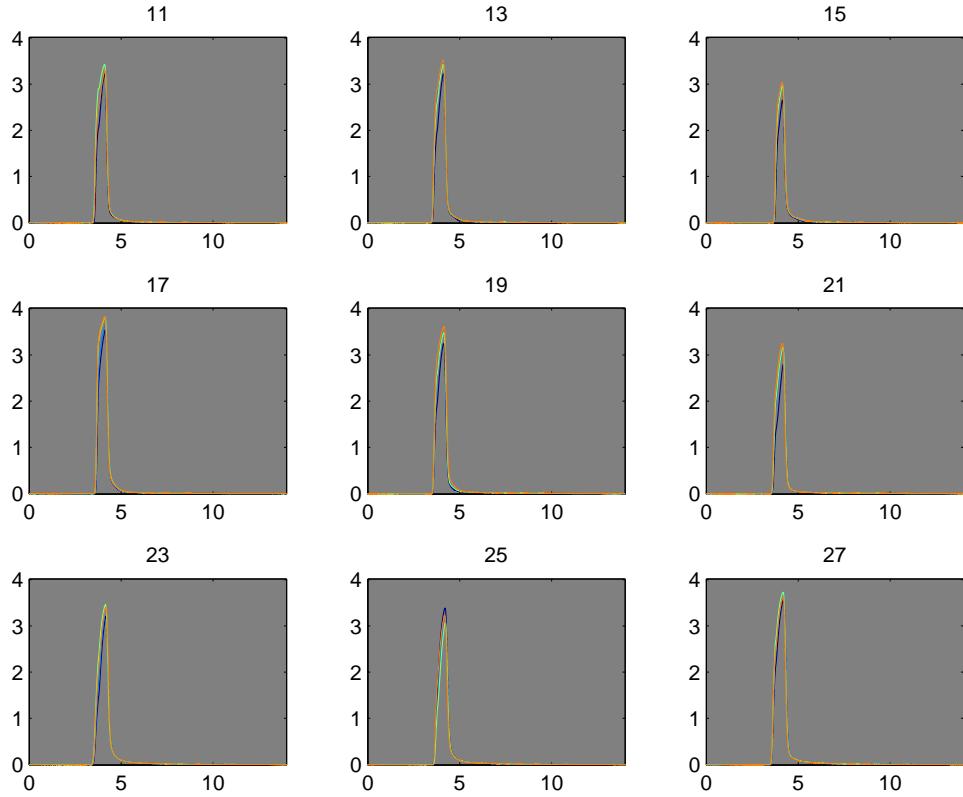


Figure 1.3: `params_100324_125249`: Replace check valve at 25 and run again (but with slightly faster ISI and no empty vial). Looks better, but the build-up of concentration is a problem.

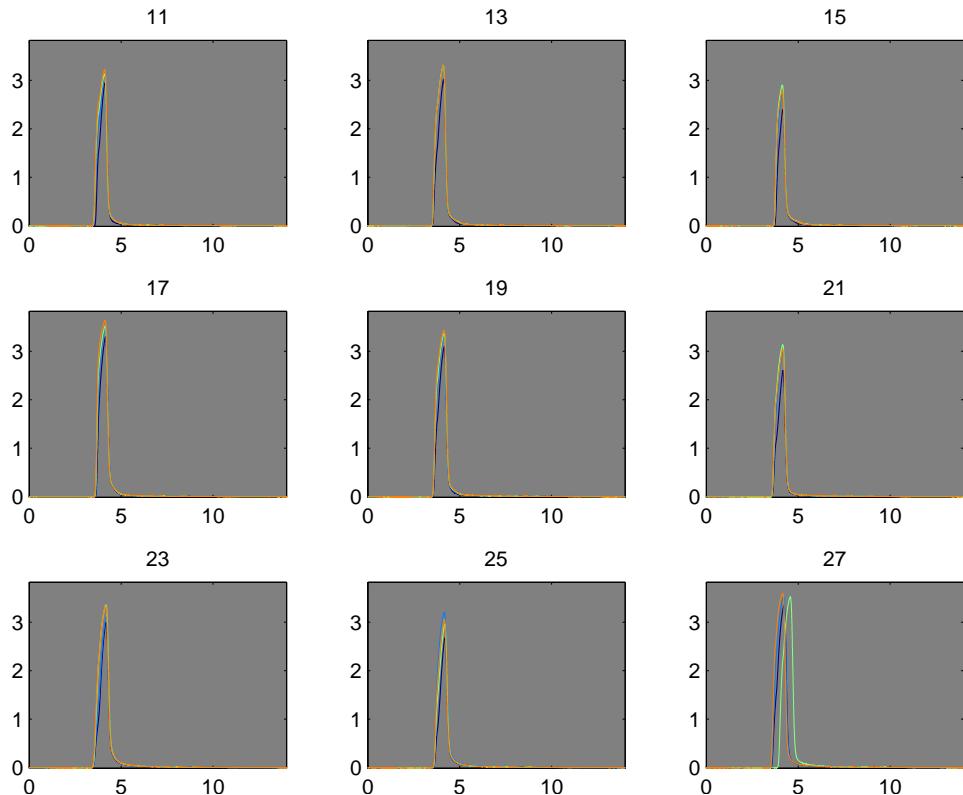


Figure 1.4: `params_100324_133823`: Let's try once more, having had the system quiet for 40 minutes. Still bad. About as bad as before.

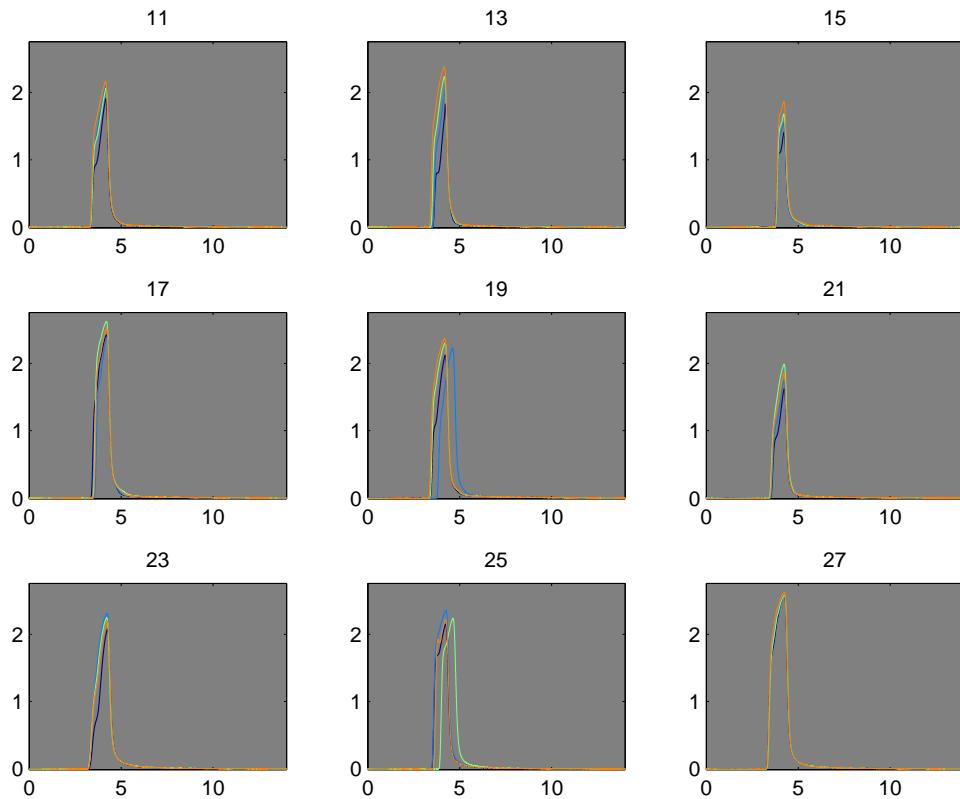


Figure 1.5: params_100324_140642:

Now we apply two changes.

1. Keep MFC switched on the whole time and flip between the empty and odourised vial. The PID signal does go up when I use the empty vial, but I don't smell anything.
2. Vial 13 now has a PTFE tube that goes most of the way down to the liquid. I am experimenting to see if this results in more stationary performance.

Run two of these with a 20 minute interval (params_100324_140642.mat, params_100324_143634.mat). Signals are also smaller. But it's not clear why that would be. It may be crap building up in the head.

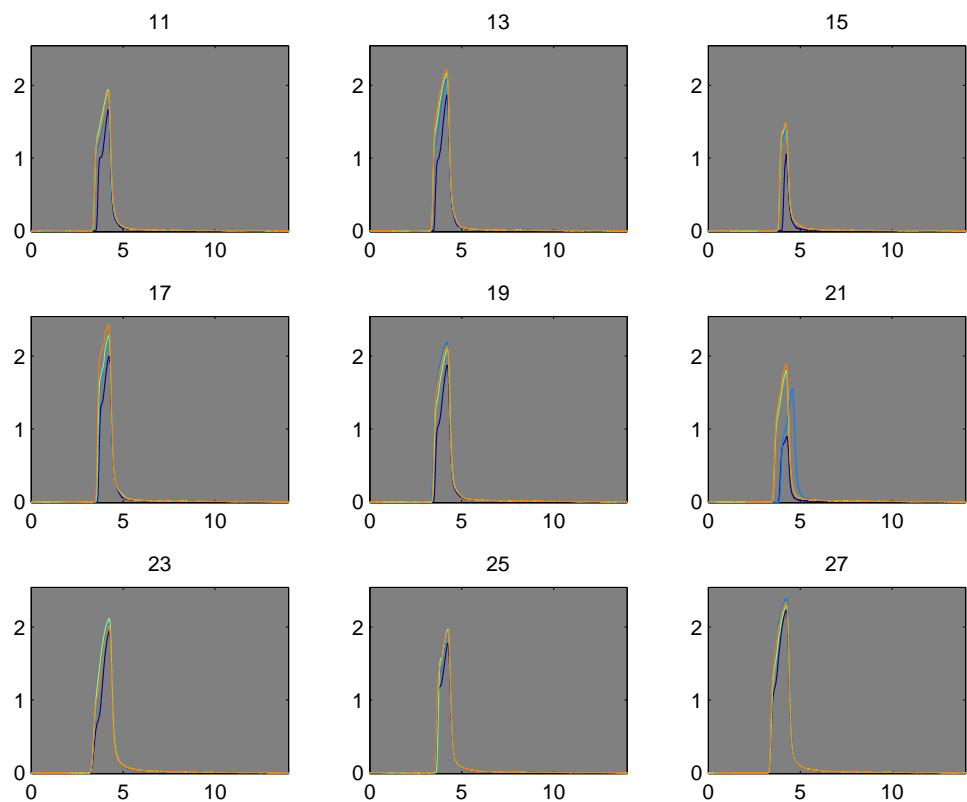


Figure 1.6: params_100324_143634:

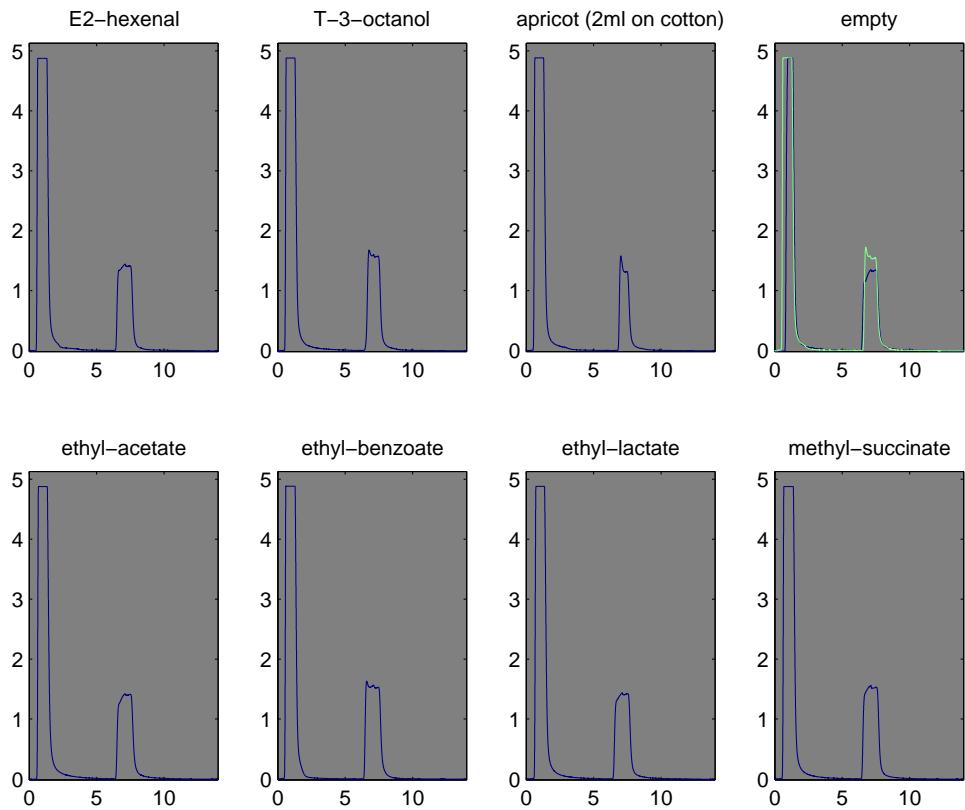


Figure 1.7: params_100324_165731:

PID went to e-room and was cleaned. params_100324_161131.mat Now it's clipping. That suggests that dirt was the reason why the signal went down earlier.

Run it again... params_100324_170457.mat (gain settings are lower) This time we deliver a 0.5 s pulse at 800 ml/min about 5 s before the "true" odour pulse. Let's see if this makes things better. Yes, it does make things better. I'm starting to suspect these weird delays are due to the check-valves because you see them less with a higher flow rate.

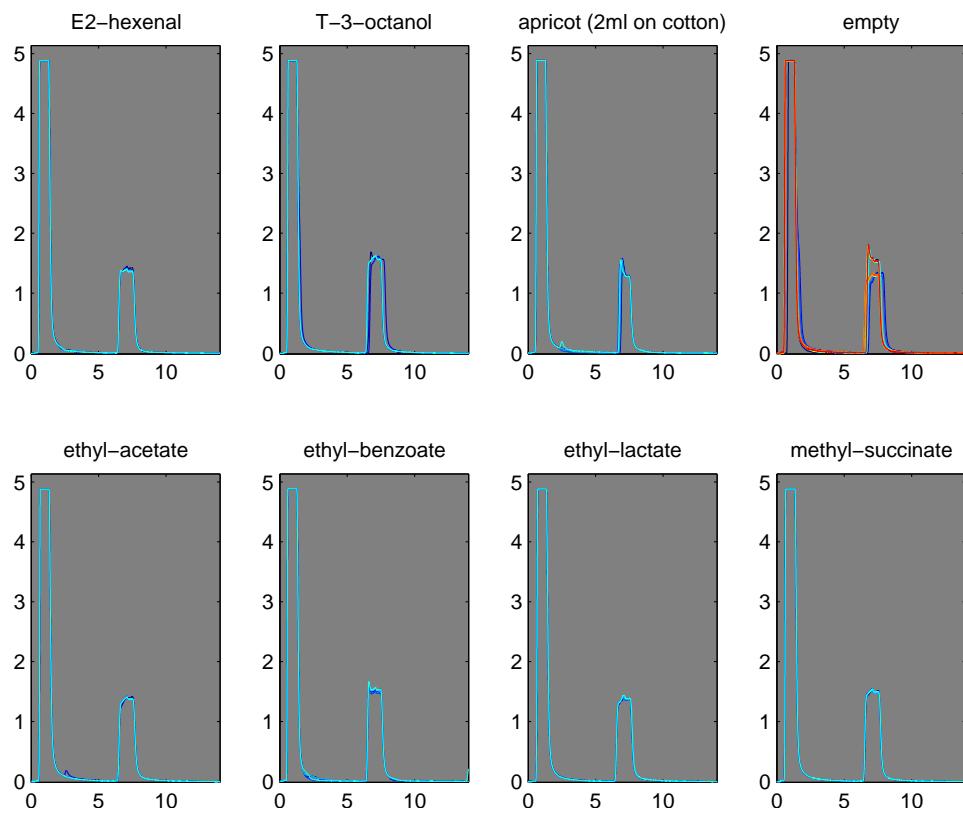


Figure 1.8: params_100324_170457:

1.2.3 25th March 2010

Set gain to x5 and bubble air through ethanol in tube 13. Bubbling air through the ethanol at 200 ml/min doesn't seem to cause crap to fly around everywhere. This is likely because the flow rate is low and we're not allowing pressure to build up. The signal is much higher from this vial but not clipping PID. We run x5 and see if it's reproducible. See params_100325_125504. I don't know why, but vial 27 is producing a much larger signal than the others.

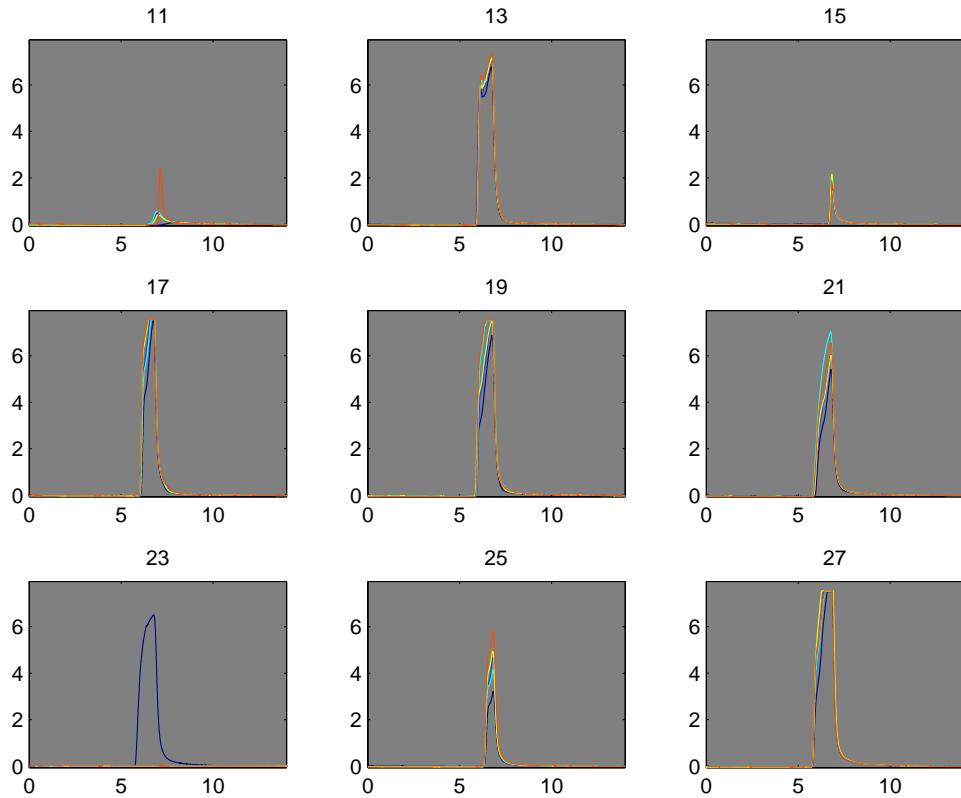


Figure 1.9: params_100325_125504:

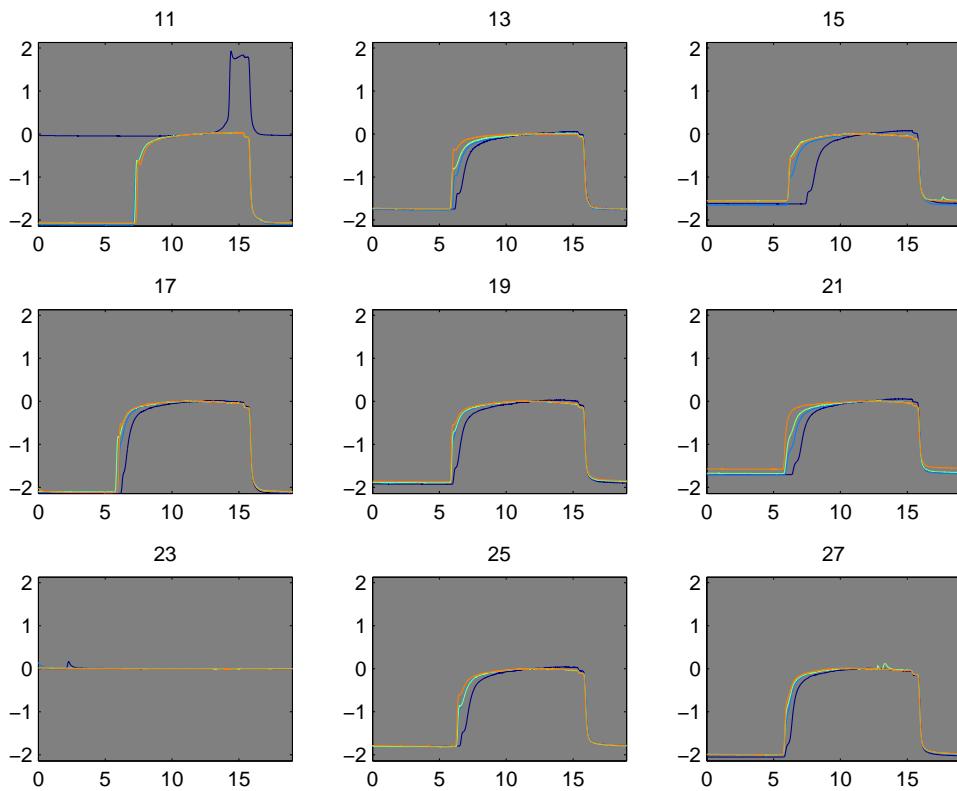


Figure 1.10: `params_100325_144139`: Now run 10 seconds of odour through each vial to see how flat the response is. I have set the PID gain to 1x, otherwise the signal saturates the amp for some vials. It's too erratic.

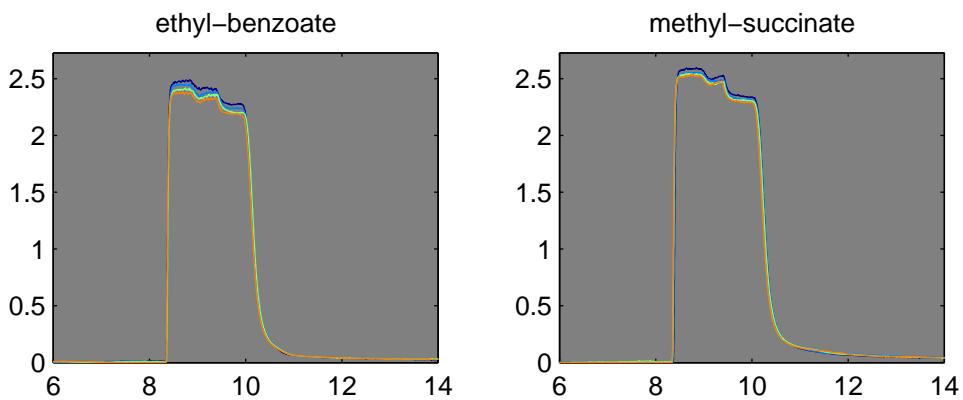


Figure 1.11: `params_100325_161650`: Because the last was too erratic I set up the old final valve with just the last pair of valves. One is odour one is clean air. The OFF step is brought about by the vial switching off so that the whole system cleans out. Doesn't look too bad

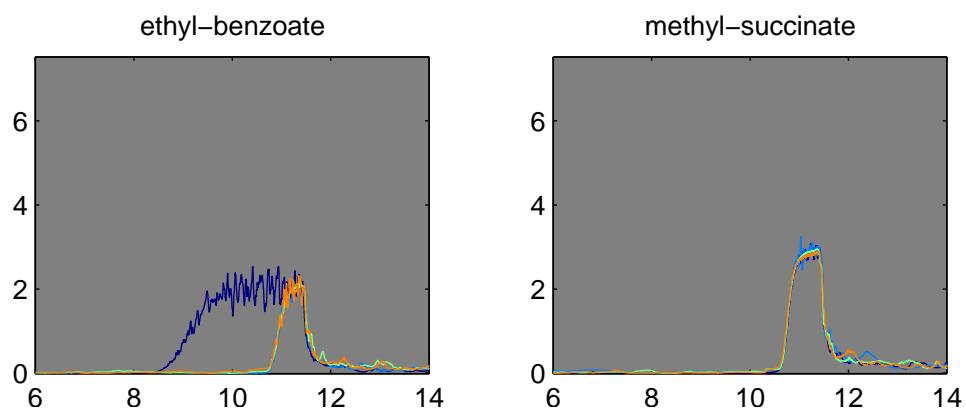


Figure 1.12: params_100325_164606: Fiddled with the code a bit. Noticed that flow through the vial goes down when we're routing it through the valve. Repeated it again and it looks really awful but I don't know why.

1.2.4 26th March 2010

Ok ,it looks like the small diameter of the final valves (1/16th inch) means that the check valves don't open right. I have put an old, crappy, check into valve 13 where we're bubbling. Bubble at 0.5 L/min for 3 secs then down to 0.2 then present. Also, I'd bubbled extensively through that vial before for testing. Looks awesomely good.

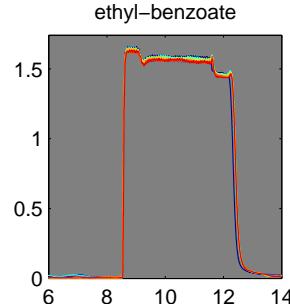


Figure 1.13: params_100326_131251: Nice!

Now wait two hours and run it again: params_100326_151344.mat The first two stimulus presentations are higher than the rest so the system does take a little time to equilibrate. Wait half an hour and we get the same problem. params_100326_154004.mat

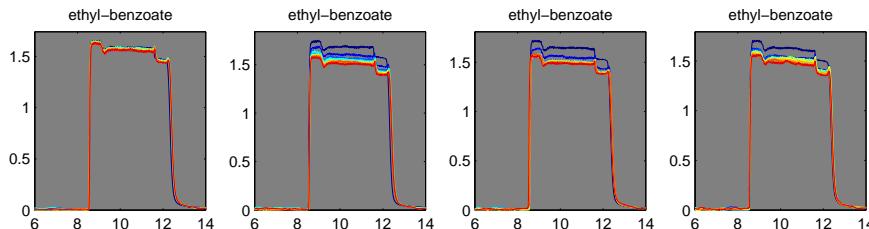


Figure 1.14: params_100326_131251; params_100326_151344; params_100326_154004; params_100326_161720;

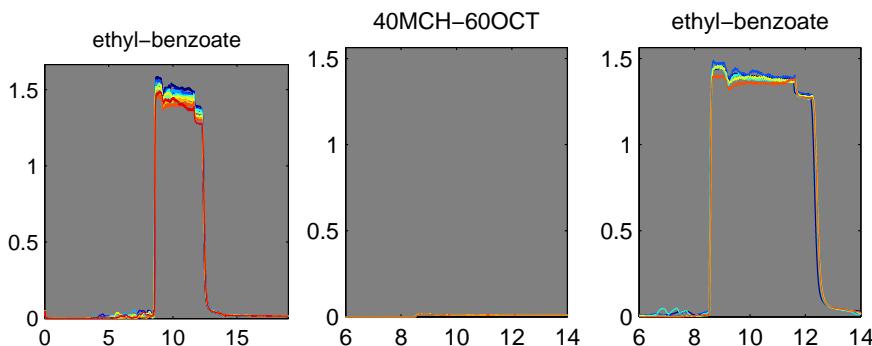


Figure 1.15: params_100326_162803 & params_100326_163150: I increased the time over which we bubble at a higher rate and that has increased variance.

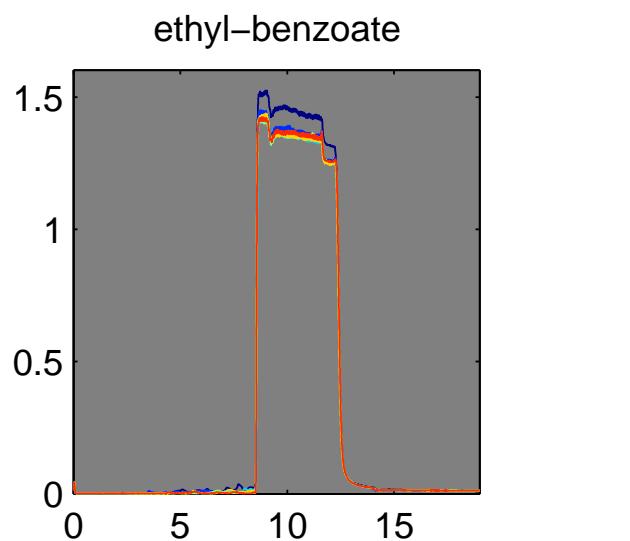


Figure 1.16: params_100326_163630:

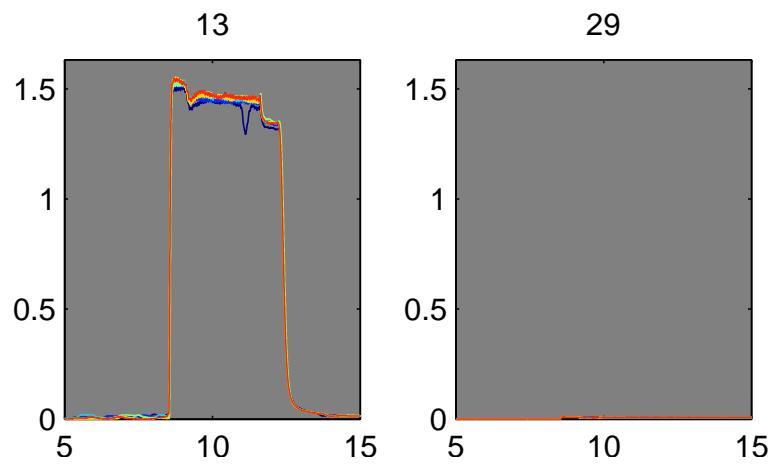


Figure 1.17: params_100326_165043: Looking better now that I set pre-presentation bubbling to 0.4

Fill V13 1/10 with ethanol and run it 15 times to see what it looks like. Eeek. It clearly hasn't equilibrated yet. I also forgot to vortex it. params_100326_171154.mat First rep was at 1x gain.

Will vortex and try again. params_100326_172130.mat That's no better. It's still going down. longer ISI may have improved things. I wonder if with a diluted odour I shouldn't be bubbling for as long. Try that. load params_100326_180042.mat

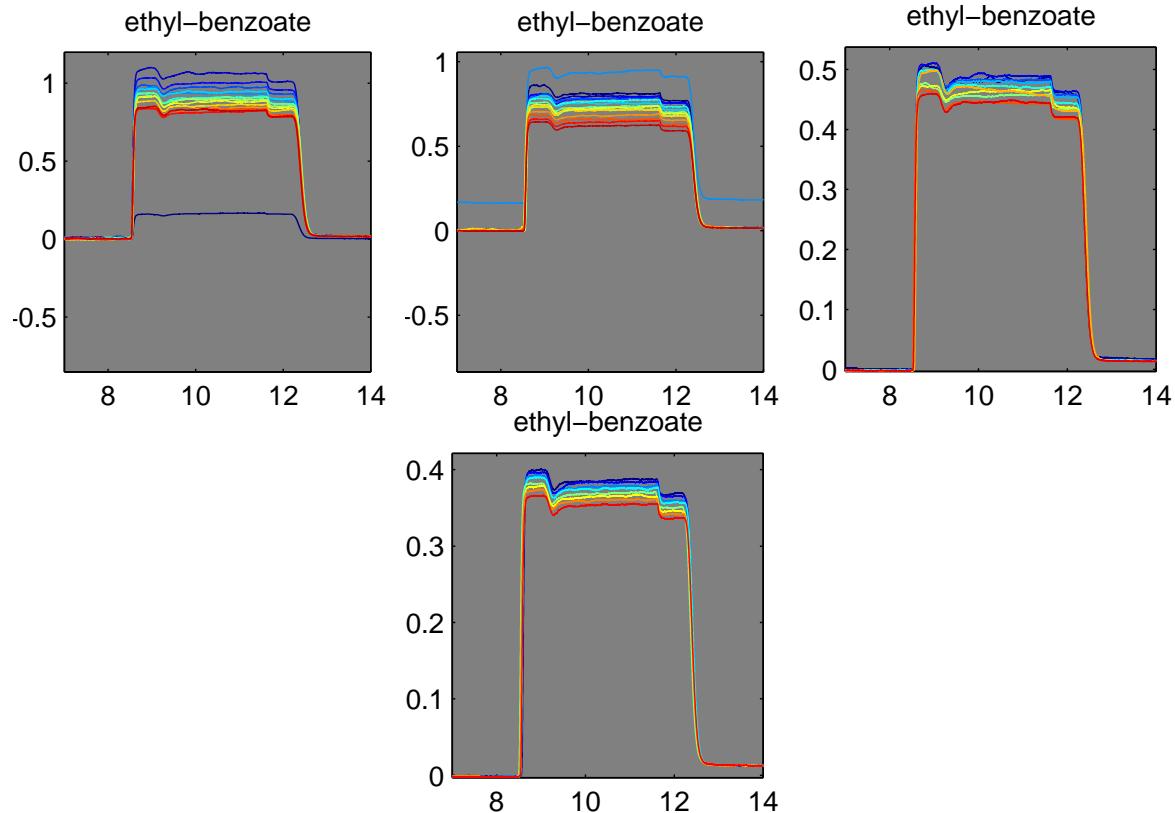


Figure 1.18: params_100326_171154, params_100326_172130, params_100326_180042, params_100326_181209.

Looking at the PID trace, it seems that 1s at 0.4 ml/min is enough to increase the odour concentration to where it ought to be. So we do 1s at 0.4 then go down to 0.25 (which is where most of today has been done) to present the odour to the fly.

The concentration is just more stable when we're dealing with pure odour. As soon as you dilute it then it starts to run down. This is because the vapour pressure decreases. With pure odour that can't happen. Go back to pure odour, I think!

1.2.5 27th March 2010

Could it be that diluting at 1:1000 will improve the problem? params_100327_154706.mat params_100327_160249.mat
No, it still goes down at every presentation. What's worse is that we see the higher level of the odour valve to be more a problem. I'll pull apart the tubing there and see if I can clean it.

One last possibility: What if it takes it overnight to equilibrate? Try the 1:10 again. No, quite the opposite: params_100327_161423.mat

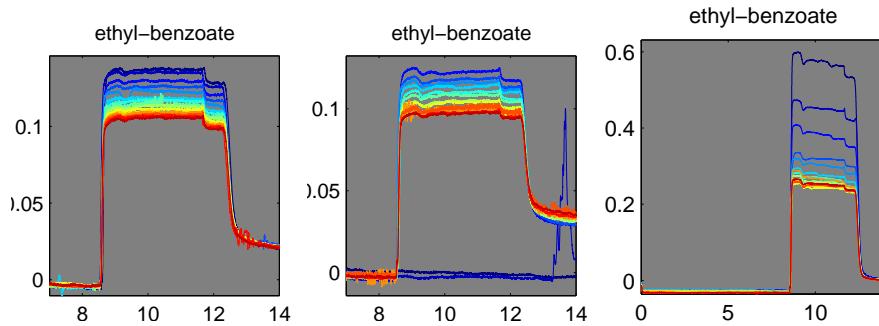


Figure 1.19: In order: params_100327_154706, params_100327_160249, params_100327_161423.

1.2.6 28th March 2010

I had noticed that the stream from the vials had a higher resting PID value than the clean air stream. Vial 13 was attached to an old style valve and a 1/1000 ethanol vial. When I replaced this vial with one containing an essential oil on cotton wool, the basal PID signal decreased. It looks like this higher signal may simply be due to the odours and can be fixed by the fancy Parker valves. Changing the clean air vial didn't alter anything and placing a clean air vial into the clean air stream didn't help either.

Today we won't use the final valve because I'm cleaning the Y. Instead, we put the PID directly onto the end of the tube coming from the odour delivery system.

I then present odour through the essential oil vial so we can evaluate it for stationarity: params_100328_152704 & params_100328_153325. We still see concentration drops on each stimulus presentation.

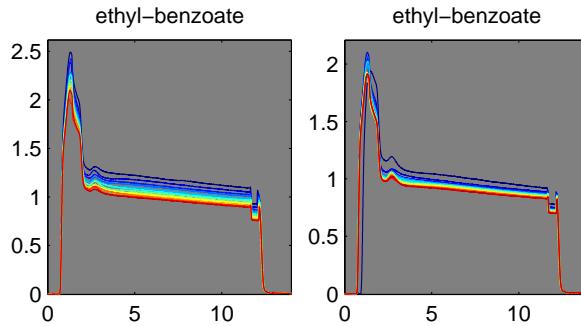


Figure 1.20: params_100328_152704, params_100328_153325

Now try with the 70/30 OCT/MCH mixture. This also goes down. It looks like there'll be no way of avoiding this unless we use saturated vapour. I think that for non-saturated vapour, params_100328_155935.mat & params_100328_160854.mat I'm probably forcing through too much air. We'd need to bubble initially so that we get rid of high-concentration head space. But then I could probably bubble at just 0.25ml/min for 7 s or so. Try this next...

It's not run out: params_100328_161932.mat

I put the pure ethanol vial back and present a few pulses to check it out again. Is it really better than the diluted odours? YES: params_100328_162845.mat

Ok... So can I shove a 1ml/min pulse into the stream and pick it up?? No. It looks like the carrier creates pressure which stops odour from entering the stream. If the difference between the streams is more than about 1/10, we get a problem.

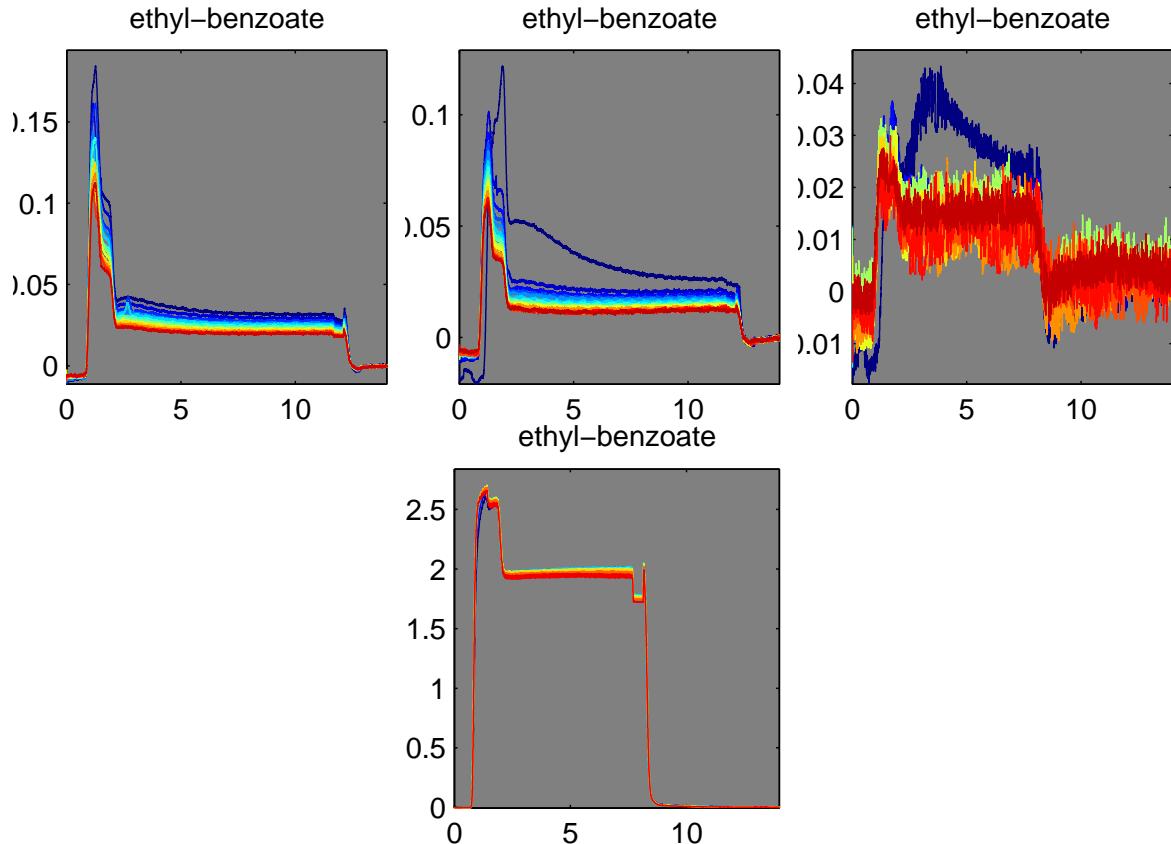


Figure 1.21: params_100328_155935, params_100328_160854, params_100328_161932, params_100328_162845.

1.2.7 29th March 2010

We have decided to run the oct/mch experiment using dilutions. Firstly, I will run ethanol though all vials and choose 6 which are best matched. Secondly, then I equalise the intensity of the MCH and Octanol with the T-maze. I will also see if using a large volume of liquid works better than a small volume. I.e. a more stable trace can be obtained this way.

Ok. We try to present each 5 times. This is 50 reps but the data acquisition seems to have halted at 34, for some odd reason. Try to work out why. Ah! It's because I started PV! Before each of the following I'm bubbling at 0.5 ml/min for 10 s through each vial in turn and then beginning the acquisition.

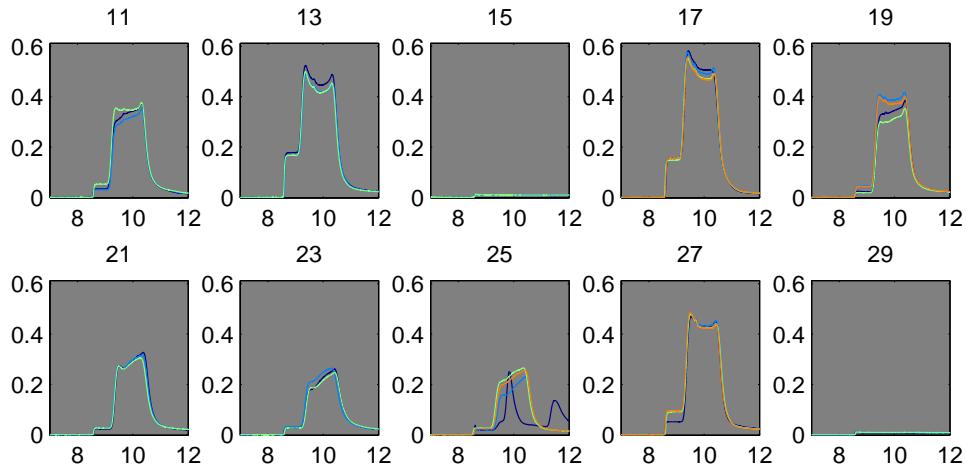


Figure 1.22: params_100329_115622:

I'm having troubling getting valve 15 to open: params_100329_122851.mat Will try blasting with 0.4 ml/min for 2 seconds before dropping to 0.25. If that doesn't work, I'll just see if I can do it all at .25. Probably have to anyway, since I think we'll need a high flow rate to get the t-maze concentrations. No, this didn't work either: params_100329_132054.mat

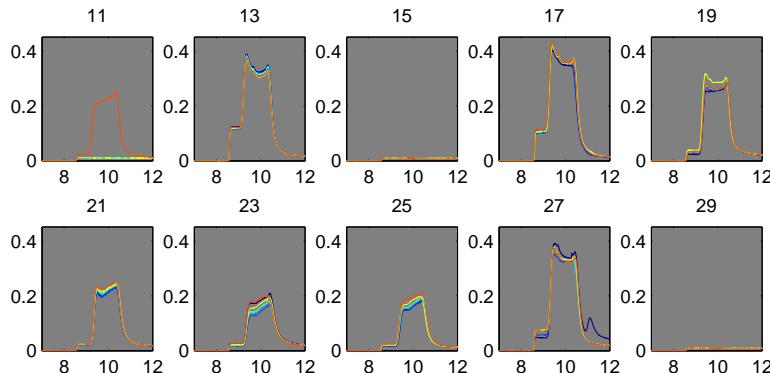


Figure 1.23: params_100329_122851:

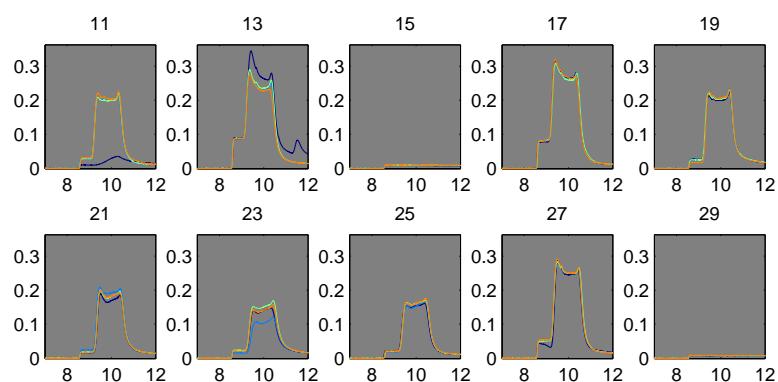


Figure 1.24: params_100329_132054:

Ok: So I will try presenting the odours at 0.4 ml/min It's nicer and more consistent at 0.4.
params_100329_134328

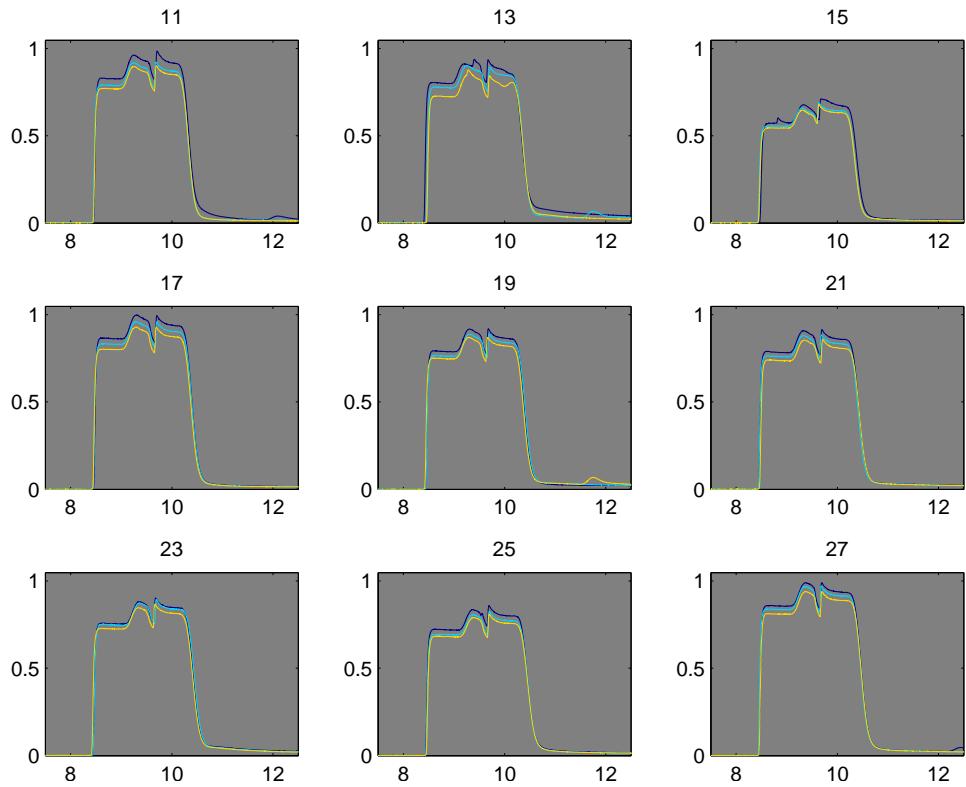


Figure 1.25: params_100329_134328:

I'll run more at 0.5 but will only run those vials that look fairly similar to each other: 11,13,19,21,23,27
 params_100329_140034: ok, but 27 is too high. Replace it with 17 and see how that goes params_100329_143349.mat
 It's not as stable as it was before. I've not been bubbling. So repeat it but bubble this time.

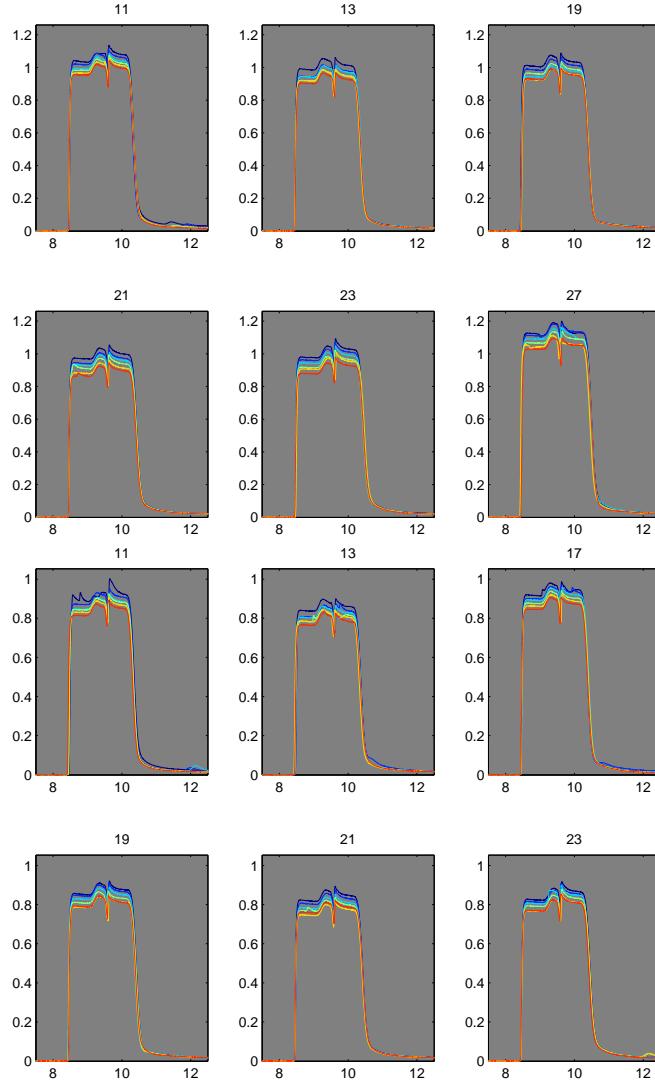


Figure 1.26: params_100329_140034, params_100329_143349

params_100329_163902.mat Still goes down over time. It's also lower than the values we got without bubbling. Argh!

Run it once more because I want to see whether the signal becomes lower than the last: params_100329_172731.mat
 Shit, the signal is going down. For all vials. Let's present x5 from 17 and another vial which hasn't been much used recently: 27

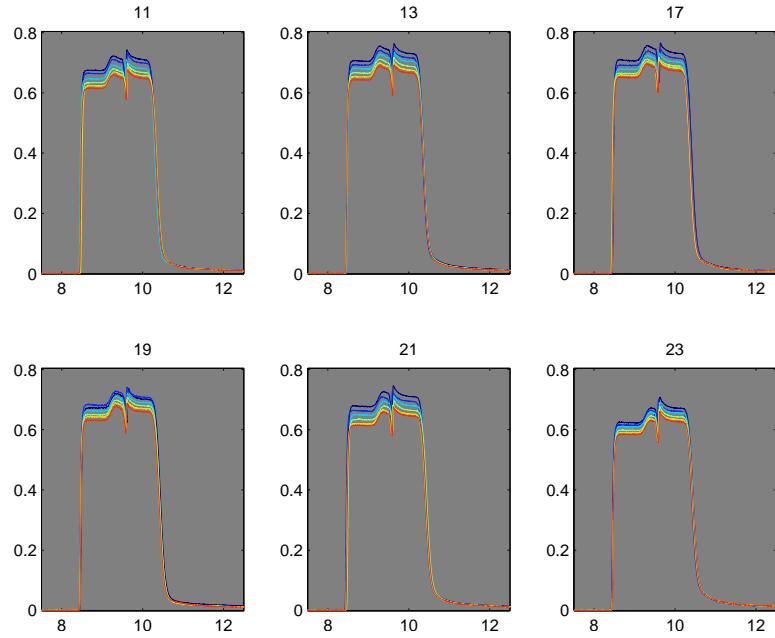


Figure 1.27: params_100329_163902:

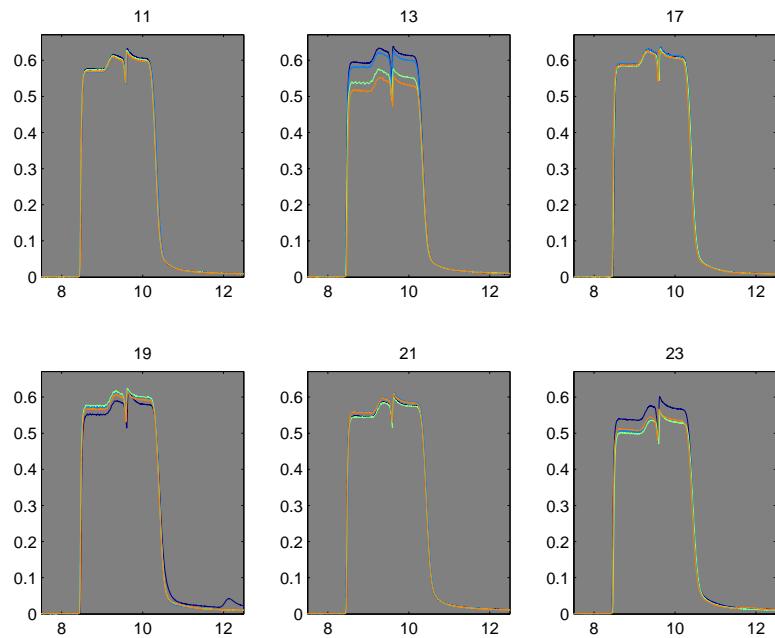


Figure 1.28: params_100329_172731:

Everything's going down (params_100329_175103.mat) since 27, which I've not been using, is also low now. I think it's the PID.... Let's clean it and try again

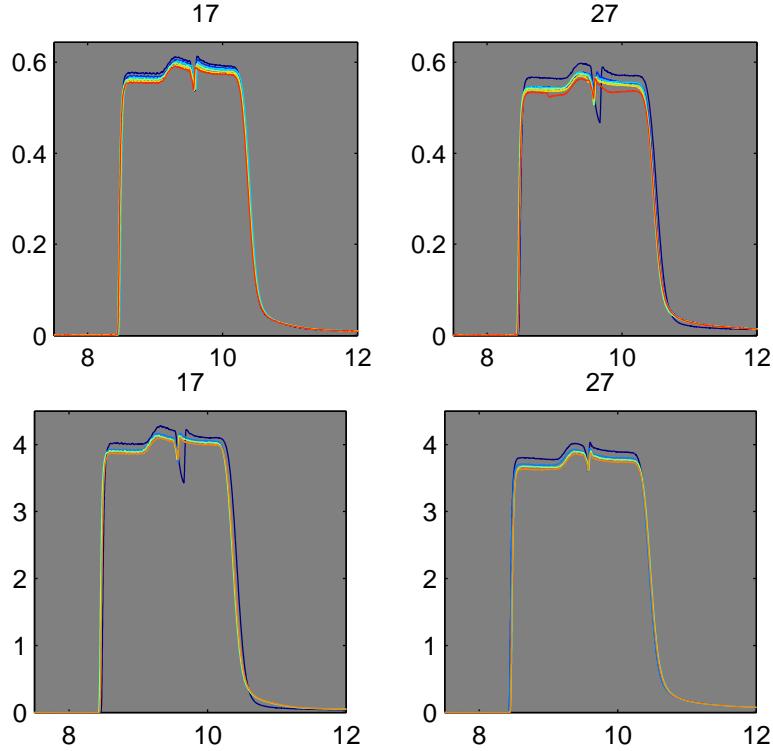


Figure 1.29: `params_100329_175103`: Everything's going down since valve 27, which I've not been using, is also low now. I think it's the PID.... Let's clean it and try again. `params_100329_181309`: Yeah, fuck. That was it! So maybe the Oct/MCH wasn't really running down after all? This wasn't flushed before recording.

I'll just do some MCH... Set PID to x1 and suction to low Set flow through the vial to 500 ml/min and stick it into #27 with the old (crappy) check valve and bubble. The MCH was freshly made today. Sod. The gain had to be at x10, because that's what we used for e-room. Do it again... `params_100329_185604.mat` `params_100329_184718.mat`

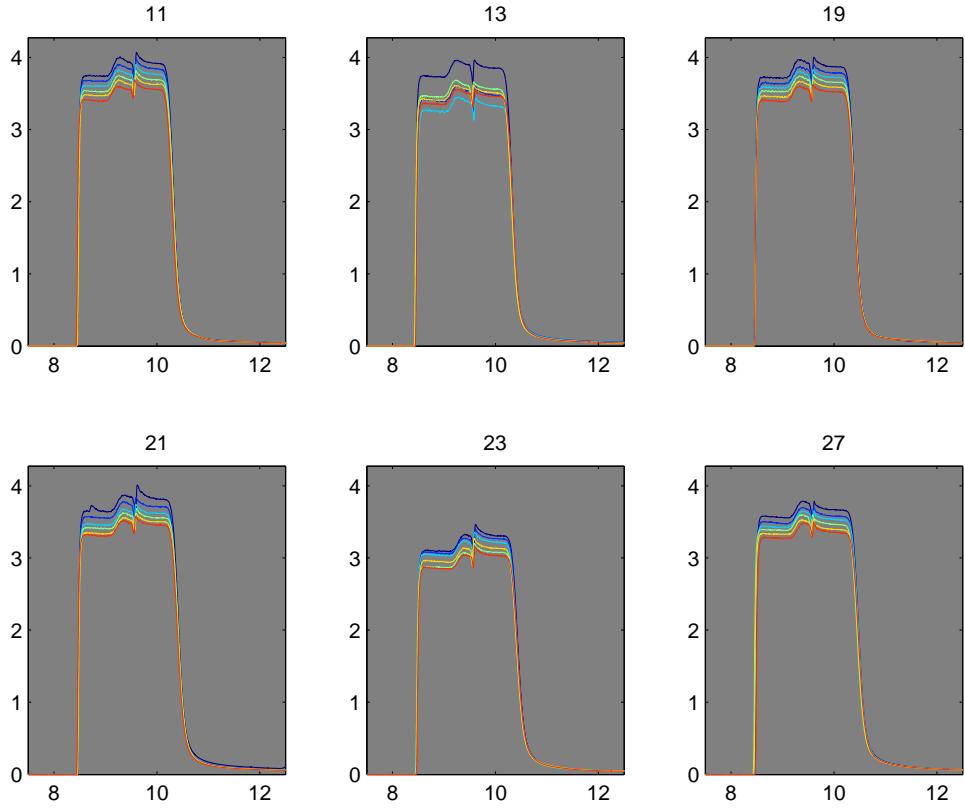


Figure 1.30: `params_100329_182227`: Let's re-run what we were doing before. *I really don't know why I got some consistently high readings before and not now. No idea. But it does look like the decrease in sensitivity is due to the PID. At least in this case.*

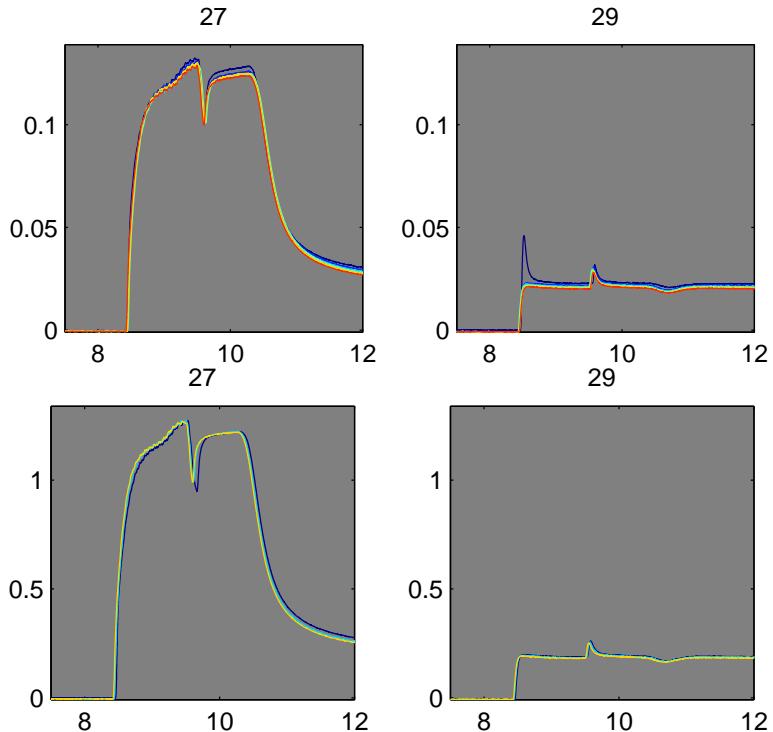


Figure 1.31: Top: `params_100329_184718`, wrong gain. Bottom: `params_100329_185604`, with the correct gain. This is too low and we're getting a valve switching artifact. I suspect because of the high flow rate.

1.2.8 30th March 2010

Clean PID again using air from the wall. Dropped the bulb, but it's not cracked and the PID lit up straight away. Flow set to low.

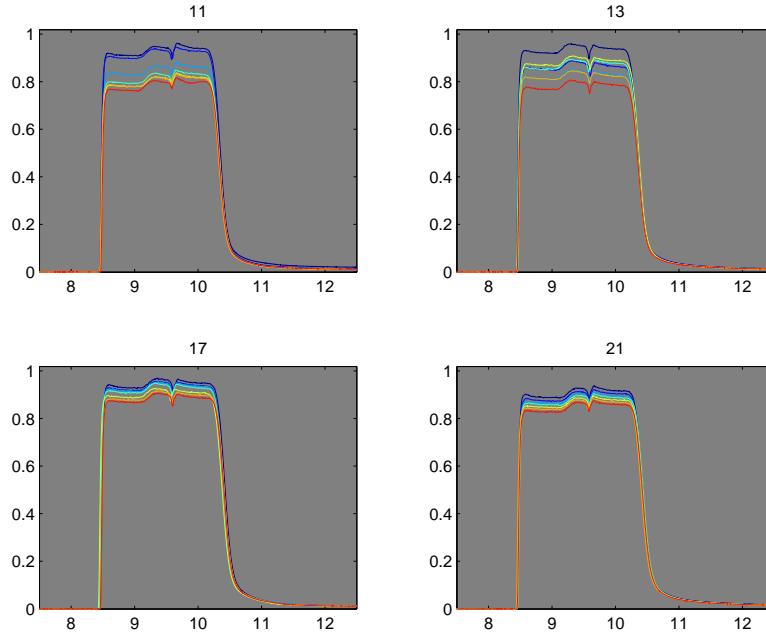


Figure 1.32: params_100330_115524: Test with ethanol from 4 of vials. 6 reps each. Bubbling through each. Odour flow is 0.5/0.5 why does it now look so bad? It was stationary before!

I played around with the relative flow rates at the vials and found that the signal at the PID is lower if the ratio of carrier to odour is not 50/50. i.e. 200 ml/min odour and 200 ml/min air is a stronger response than 400 ml/min odour. I do another run, therefore, with the valves we'll want to use and with 0.35 l/min going through both carrier and odour.

So now, all I need to do is equalise the concentrations. First we present a load of stuff through #27, since we weren't doing this and we need 6 vials.

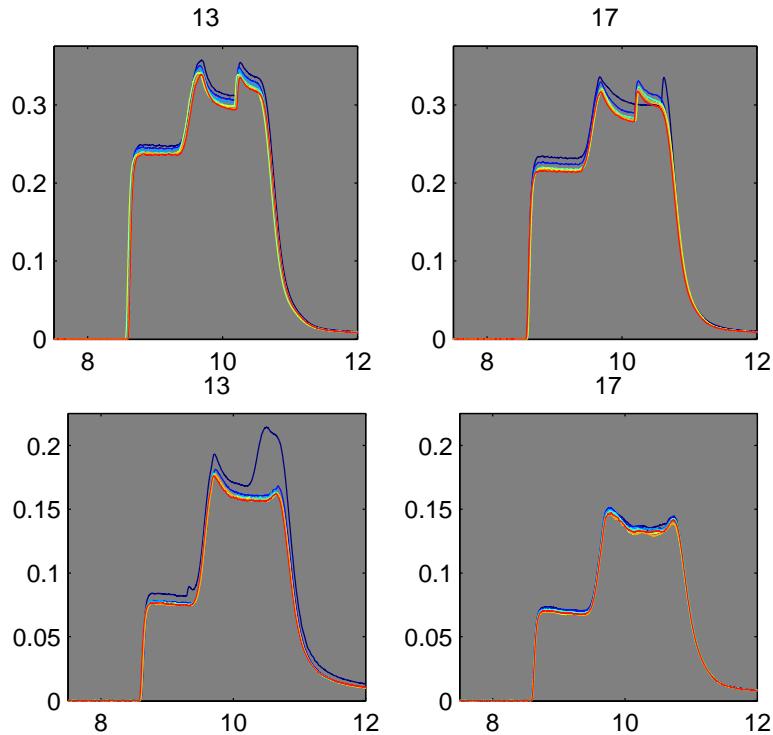


Figure 1.33: params_100330_121140: Try dropping down to 0.25 ml/min. Yeah... that looks better. And again at 0.2: params_100330_123018.

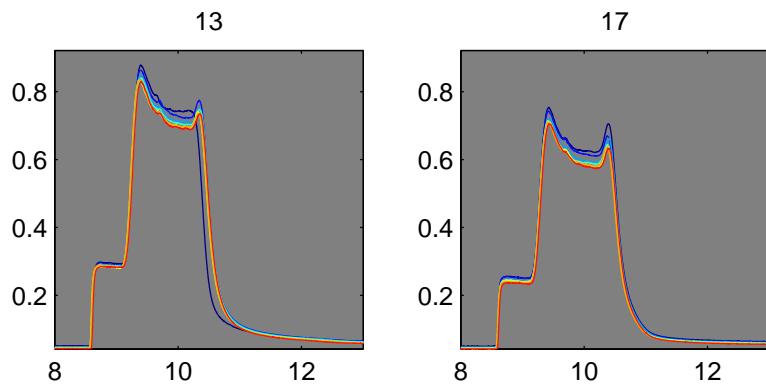


Figure 1.34: params_100330_162733: PID was running all afternoon. Cleaned it again. Wow, the signal went up. This thing needs regular cleaning. I am running it again with the flow at 0.2 and total flow at 1 (the previous two in the preceding fig were actually at a higher total flow).

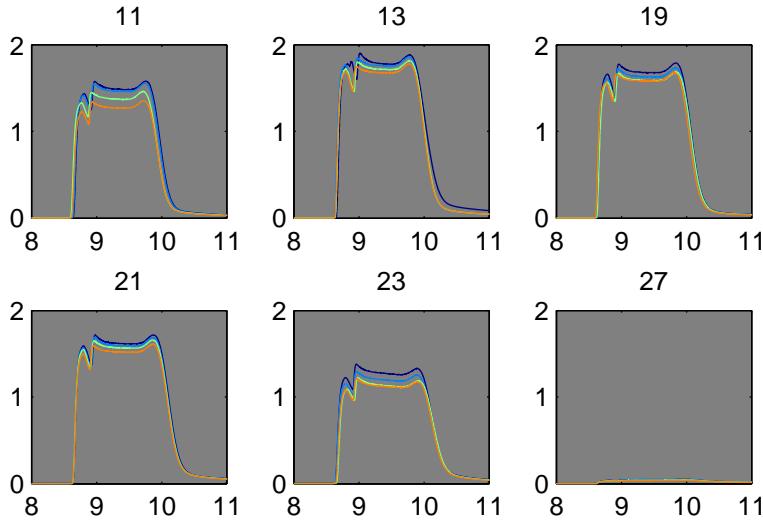


Figure 1.35: `params_100330_174954`: #27 is actually an MCH vial which we won't end up using. The signal goes down over time but this may be because of the flushing procedure. It flushes through 0.5 l/min but the odour is less than that. Perhaps it takes a while to settle. I therefore run it again, below, without the flushing.

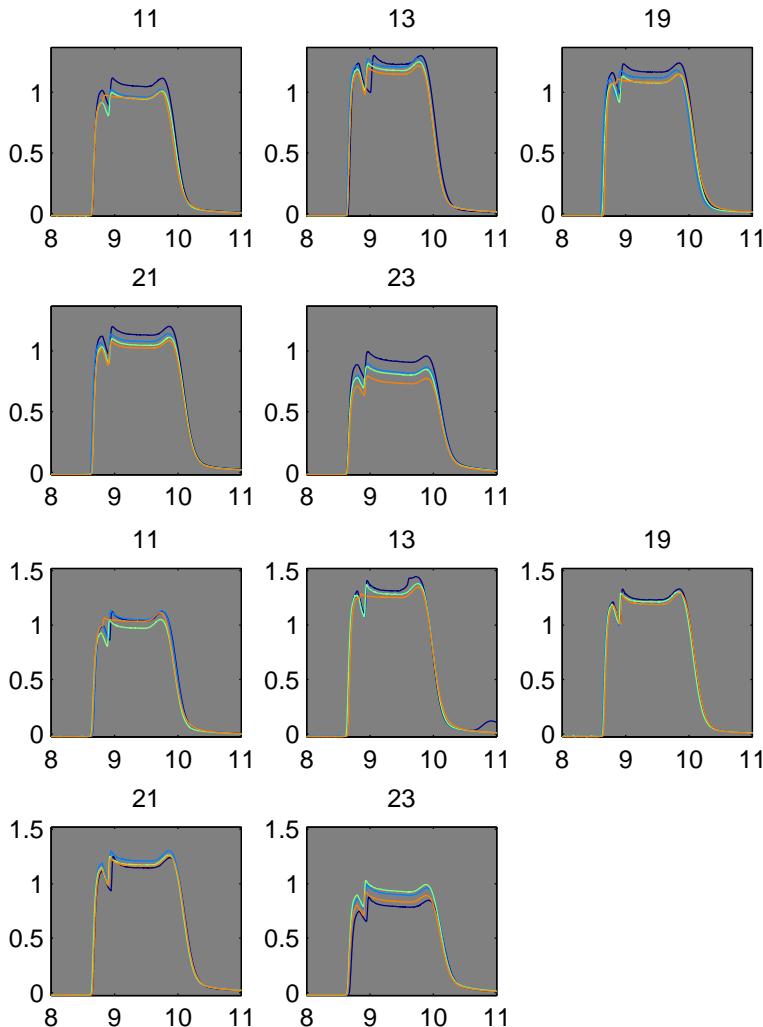


Figure 1.36: `params_100330_180600`: Still goes down. How about we flush at the same rate that we'll be presenting at? Also, clean the PID again. The result of this is in the bottom two rows: `params_100330_182240`: now in some vials it's going up.

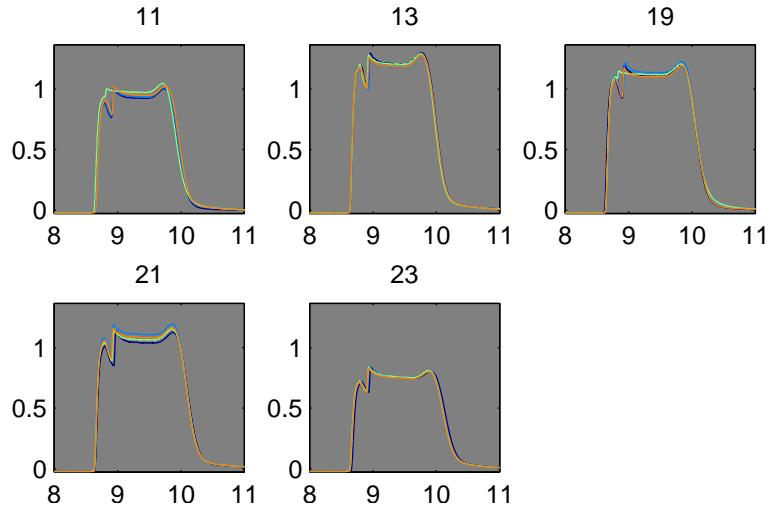


Figure 1.37: Deliver once more with no PID, see if we can equalise everything further. Then record: `params_100330_185432`. This is looking better. So it takes it a looong time to equilibrate and it's a BAD idea to be running air through at different odour concentrations.

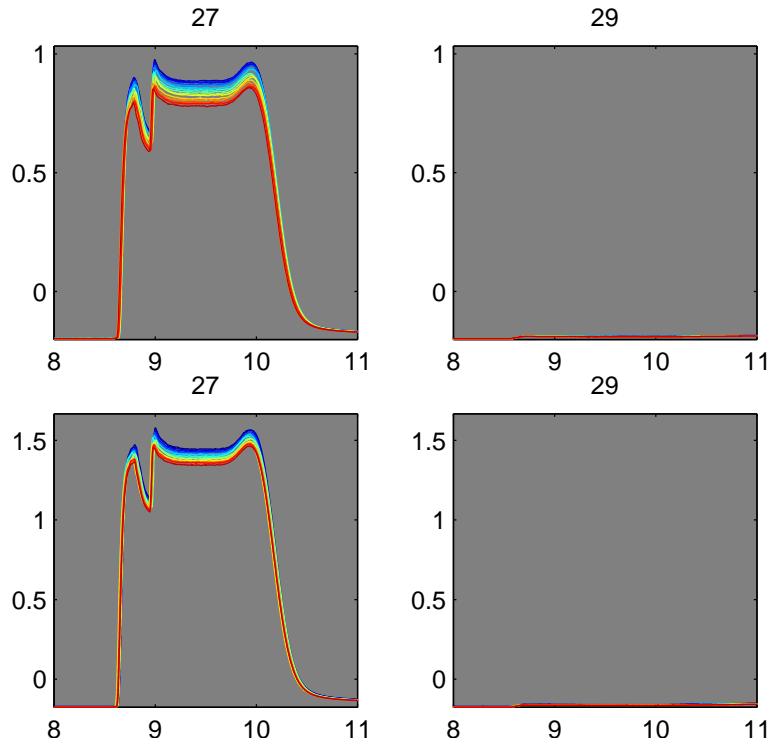


Figure 1.38: Top: `params_100330_192526`. Clean the PID and repeat Bottom `params_100330_194547`. Equilibrating takes a loong time!

1.2.9 31th March 2010

Blast air through all the check valves. Now flush the 7 valves we'll use for 15 s each. Then present stimuli. Do everything at 0.35 ml/min Ooh. They look totally different. Very smooth traces now.

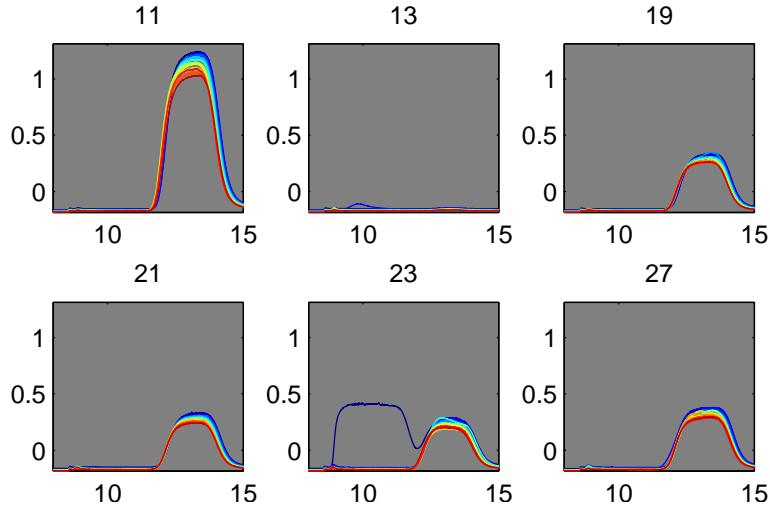


Figure 1.39: params_100331_110932: This is a disaster. The final valve is switching at 8s then nothing happens until 2 seconds later. 11 is way bigger than the others and 13 doesn't respond at all.

Ohh! Check valve 13 was disconnected so the system wasn't pressurised.

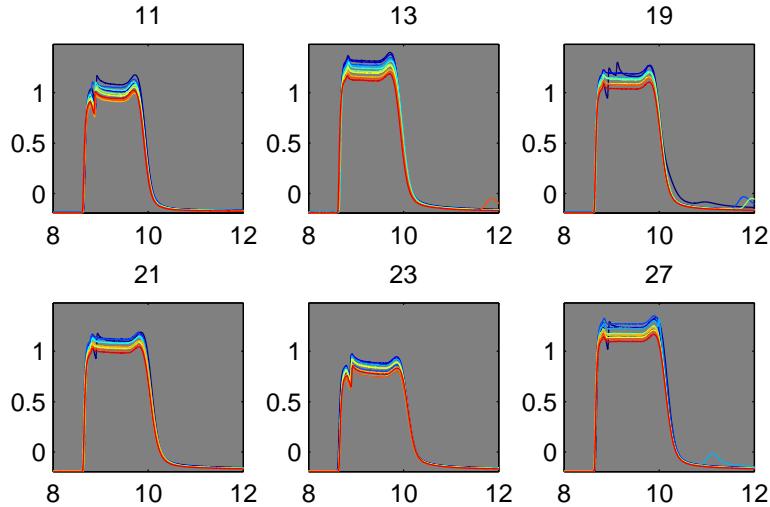


Figure 1.40: params_100331_115521:

Replace the check valve in #23 and then flush/run for 3 reps.

Why are the signals from different vials of different strength? I'll check the flow rates from all the vials. In the process of doing this, I found a bug in the code: when the system was being asked to go to valve 29, it was failing to do so. Instead it just shut all flow to the valves. I am therefore now running it again, with the bug corrected. Ok, so the flows. They're very similar:

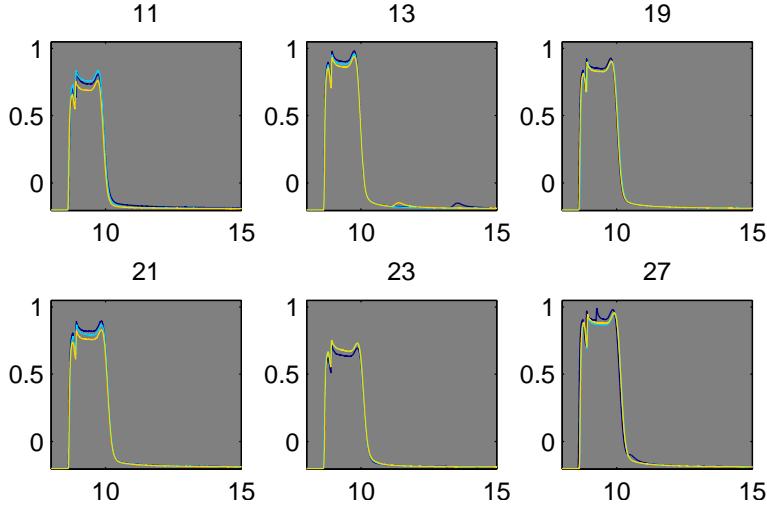


Figure 1.41: params_100331_131419: It's still lower.

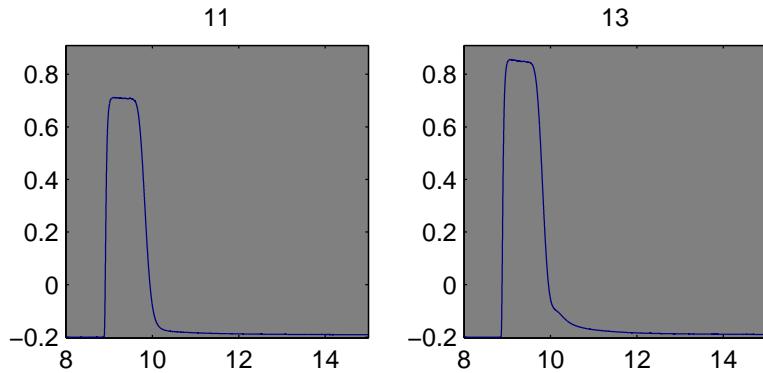


Figure 1.42: params_100331_132312: We can get much nicer curves that are closer to 1s in duration if we switch the vial off 100 ms before the odour valve opens. The stimulus duration parameter doesn't do anything in the delivery code in this context.

Valve	flow
29	0.95
27	0.93
25	0.95
23	0.94
21	0.93
19	0.93
17	0.90
15	0.96
13	0.91
11	0.96

This was measured as follows: Set the carrier flow to 1 l/min. The flow meter does

indeed register that this is at 1 l/min. The odd thing is that if I set the vials to be 1 l/min, I only get 0.5 l/min. **Hmmm. Is there a leak or something I don't understand.** The flows seem additive when both controllers are on. When I measure the flow at the odour MFC, I get about 950 ml/min. The carrier is about 1050 ml/min. So maybe I should service these things? When I measure after valve 31, I get about 800 or 900 ml/min. So There must be a leak up there. I'll ignore it for this week, since I need to get data. But when I build the new system, *the first thing to check for is that the flows add up correctly.* So build the system with empty vials first. Regardless, I ask what flow through the vial do I need to achieve a total flow of 1,500 ml/min. These are the values in the table. They appear very similar for most vials. I choose the vials which are most similar and run the thing again. Let's see what it looks like now.

So first I replace all vials with empties (wide bore outlet tube) and flush: pump 1 l/min through

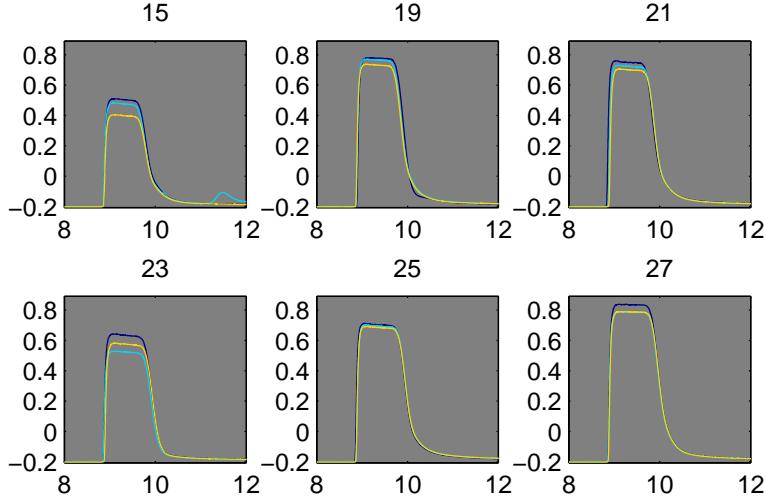


Figure 1.43: params_100331_140949: Don't know why 23 and 15 look so bad. Only they're similar to each other. I say I go ahead with oct and mch in these two then the rest of the odours can be other vials. It's worth noting that the stimulus latency is supposed to be at 8 s. It's clearly not. So that is worth fixing.

each vial for 15 s each. Keep doing this whilst I prepare the odourants. **Ah!** I found that some vials smelled quite strongly of odour. At first I thought it was that the tubes hadn't been cleaned properly downstairs. The tubes are now being boiled in water and ethanol. But then I discovered that the inlet check valves smelled quite strongly. So now those are boiling too. I changed most of them (apart from tubes 21, 23, and 25, because these were the least bad and I've run out of untainted check valves). There is still some signal and I'm worried that it's coming from the valve. There's really nothing I can do now. I'll just keep flushing and use the same vials I had originally planned.

Let's try to do a recording

I'll begin by shoving 10 ml of the 1.5:1000 MCH into V23 and recording 10 reps of that (no flushing). PID gain is x10 and the suction rate is on low (900 ml/min). These were the settings used for measuring the odour concentrations in the RoboTrainer (params_100331_161748). Now clean the PID and do that again (params_100331_164630).

This MCH vial has been used in other recordings over the last few days (see above). So how much of it has *really* gone down? To answer this, we clean the PID again and replace the MCH with new stuff. Now record.

The PID response appears to be very weak. I don't trust it. Even with the nozzle inside the vial's head-space the signal is weak. I wonder if maybe the bulb is damaged and has sprung a leak? I want to do the experiment. So sod it. I'll just fill it all up with the T-maze stuff and go for it. Actually, later I read the manual and realised that the bulbs can be cleaned with methanol. Did this and the signal has now shot up.

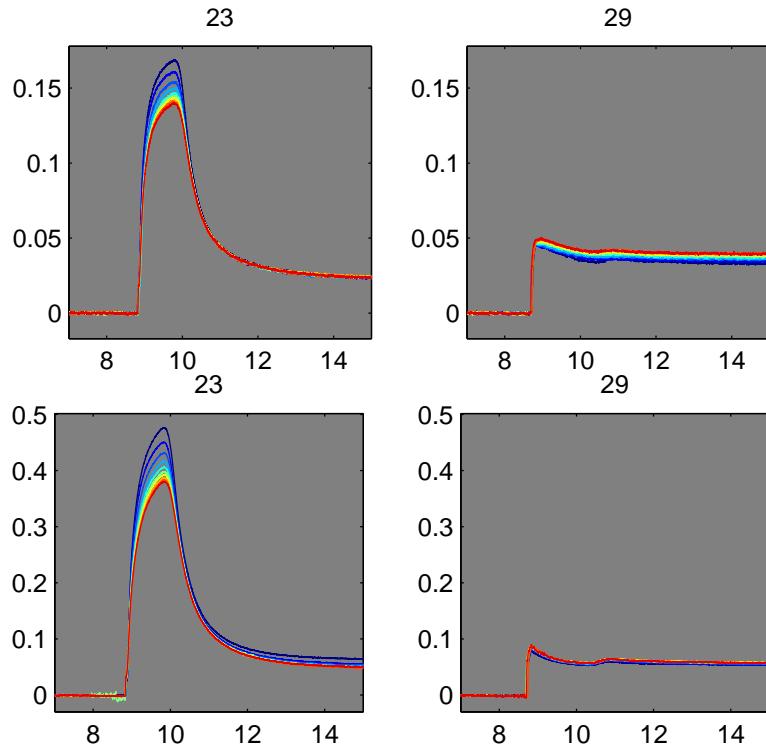


Figure 1.44: Top row: `params_100331_161748`; bottom row: `params_100331_164630`. The lower recording when I'd cleaned the PID is so much bigger! I really think that a (large) part of the decrease that we see in the response could be due to the PID and not true reduction in concentration.

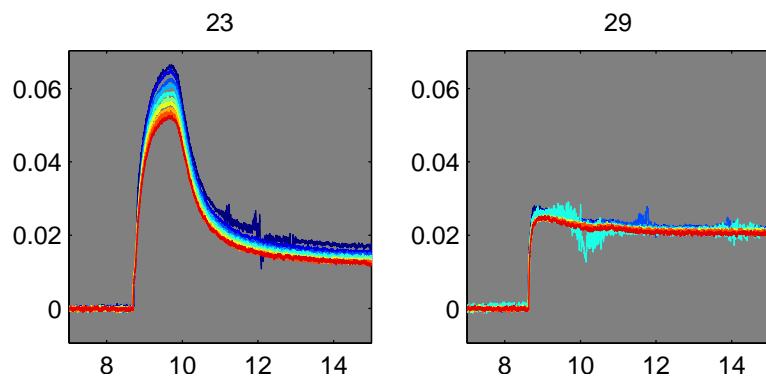


Figure 1.45: `params_100331_172029`: Great. That's all I needed. Now the signal's gone down.

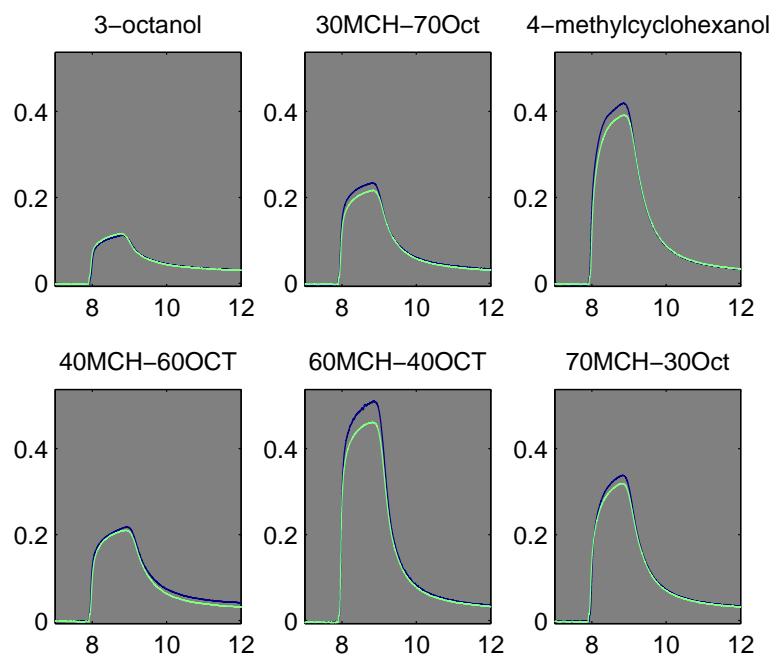


Figure 1.46: params_100331_195540: